The Yale Journal of Biology and Medicine

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THE YALE JOURNAL OF BIOLOGY AND MEDICINE

Founded in 1928

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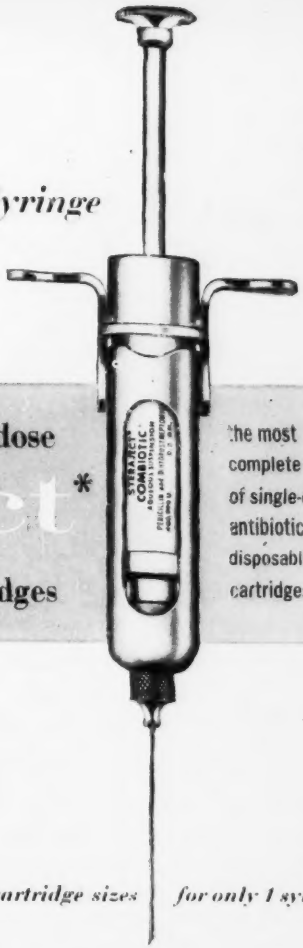

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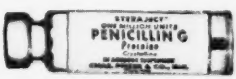

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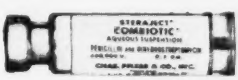

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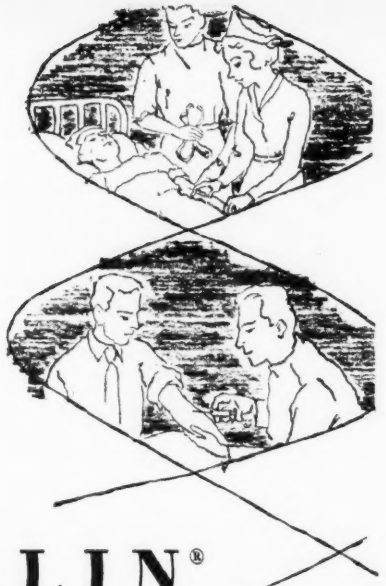

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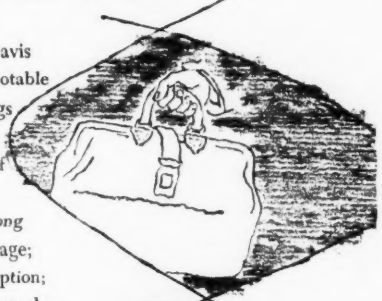

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GEORGE HATHORN SMITH

1886-1952

With the passing of George Hathorn Smith, Yale has lost a mellow scholar and teacher, and this Journal its founding father and devoted guide until his tragic illness made it impossible for him to continue as Editor-in-Chief. Many were the lessons of his editorial wisdom, of his skill in trimming the obscuring foliage from words and pictures, and of his incisive but never unkind criticism. George Smith said, "The only thing I am anti, is anti-anti." Such a man will be sorely missed. But the telling of the fulness of the qualities that made him both great and beloved will be reserved for a special issue in his honor.

THE BOARD OF EDITORS

THE TWENTY-FIFTH ANNIVERSARY OF THE YALE JOURNAL OF BIOLOGY AND MEDICINE

Some Notes on Its History*

ELIZABETH H. THOMSON

With this issue *The Yale Journal of Biology and Medicine* enters its twenty-fifth volume. Succeeding generations of medical students have been responsible for much of the work that has gone into its six issues a year since the first in 1928, but the inspiration, the continuity, the cohesiveness of purpose and effort which have brought the Journal an international reputation as a scientific publication were supplied from 1928 to 1949 by its Editor-in-Chief, Dr. Geo. H. Smith, Professor of Immunology. During these years Dr. Smith was loyally assisted by Miss Emma M. Armonath.

Subsequent Editors—Dr. John R. Brobeck, now Professor of Physiology at the University of Pennsylvania, and Dr. Max Taffel, Associate Clinical Professor of Surgery at Yale—have endeavored to maintain the standards of excellence which had been set by Dr. Smith and to carry the *Journal* on in the broad and liberal spirit in which it was conceived and by which it has been guided during the first quarter century of its existence. In this they have been ably assisted and supported by a board of Consulting Editors[1] and by a Board of Editors drawn from the faculty of the Medical School and the departments of the biological sciences of the University. The student editors, whose active participation in the work of producing the *Journal* makes it unique among medical journals, have been a fluid group, editors usually being elected during their first year to serve throughout their medical school careers. A quarter of a century is not a long life compared to that of some journals, but for a publication whose editorial board has been constantly changing and whose financial state has always been precarious, it is a respectable age. The time and effort which Dr. Smith devoted to nurturing the *Journal* through difficult years have fashioned for him an enduring and worthy monument.

The circumstances surrounding the founding of the publication are well described in the report for 1928-1929 of the Dean of the Medical School, Dr. Milton C. Winternitz, who has given support and encouragement to the undertaking from the beginning:

> One important project that may be considered beyond the scope of what is usually included in the curriculum has been most successfully inaugurated this year; namely,

* From the Department of the History of Medicine, Yale University School of Medicine.

[1] Dr. Herbert Thoms has served in this capacity since the inception of the *Journal*; Drs. Harold S. Burr, Creighton Barker, Samuel C. Harvey, and Milton C. Winternitz have also served for many years.

the publication of the *Yale Journal of Biology and Medicine*. It may be remembered that the *Yale Medical Journal*, a very successful publication for some years, was discontinued in 1912, and, from time to time since, both students and faculty have expressed the hope that it might be revived or that some other publication would fill what was deemed to be a growing need. The enlarged School, its affiliation with the biological sciences and the many activities now being pursued, make it impossible for an individual to have timely and accurate information of many of the enterprises with which all should have a general familiarity. Further, the educational value for a limited number of students, derived from service on the editorial board of a supervised publication, cannot be disputed. Therefore, when a considerable and representative number of students, a year ago, formally requested the coöperation of the faculty in launching such a publication, it was seriously considered, the approval of all concerned was obtained with the qualification that the University assume no financial responsibility, and the plans were consummated. The interest and experience of Professor George H. Smith are largely, if not entirely, responsible for the elaboration of the plan and for the well-merited, cordial reception of the six issues of the year. Dr. Smith, as editor-in-chief, has associated with him two other members of the faculty and a group of students of the School chosen on a competitive basis and including men from both the clinical and so-called preclinical years. The actual work in connection with maintaining the journal is, theoretically at least, largely performed by the students. Its objectives are to provide an incentive to the student for preparing scientific material for publication; to familiarize all concerned with the progress of the many different enterprises which are fostered by the School in its affiliation with other divisions of the University and with the clinical institutions; and to provide an historically valuable record of the institution and its work. Although the original plan was full of promise, the achievements of the year have exceeded expectations. The *Journal* may even succeed in balancing its books.

In December of 1928 Dr. Smith received a letter from the Professor of Surgery at a medical school some hundred and forty miles northeast of New Haven which read:

Dear Smith:

I am delighted to see the first number of the Yale Journal of Biology and Medicine. Please put me down as a subscriber. Much power to your elbow.

I presume that is a silhouette of Nathan Smith [on the cover],[2] peace to his ashes.

Always sincerely yours,

Harvey Cushing

Since Dr. Cushing had been endeavoring for about fifteen years to arouse interest in the founding of a similar journal at the Harvard Medical School, he took this occasion to make another attempt and as a result Dr. Smith wrote him on November 20, 1929:

My dear Dr. Cushing:

I want to thank you very much for the kindly way in which you refer to the YALE JOURNAL OF BIOLOGY AND MEDICINE in your annual report of the Peter Bent Brigham Hospital. It naturally gives us much pleasure to see such favorable comment.

---

[2] The silhouette of Nathan Smith, Professor of the Theory and Practice of Physic, Surgery and Obstetricks when the Medical Institution of Yale College first opened its doors in 1813, still lends distinction to the *Journal's* cover.

May I add that we would be very proud to list you among our contributors since I am sure that our experiment in publishing the Journal would gain much support if we could publish something under your name. I might also add that I am pleased to recognize that you are one of the few individuals who realize the objective of the Journal.

Very sincerely yours,
Geo. H. Smith
Editor-in-Chief

Although in his reply Dr. Cushing assured Dr. Smith that he hoped some day to send him something to publish, it was not until 1935, after he had joined the Yale faculty as Professor of Neurology, that a paper of his did appear in the *Journal*. This, "Psychiatrists, neurologists and the neurosurgeon,"[3] was followed by two more—"William Beaumont's rendezvous with fame"[4] and an account of "Ercole Lelli and his écorché."[5]

The first issue of the *Yale Journal* contained 64 pages which were distributed as follows: 21 devoted to papers, 11 to a section called Comments containing news of people and new developments at Yale, 13 to Events, which consisted of abstracts of papers presented to the Yale Medical Society and at the Fourth Clinical Congress (a Connecticut institution which meets yearly in the early autumn), and 18 to Abstracts and Reviews. The sixty-fourth page carried four advertisements.

The papers included one by Dr. James D. Trask of the Department of Pediatrics entitled "Comparative immunology in pediatrics"; one on "Problems concerning the origin and development of the neural crest and cranial ganglia in the vertebrates" by Dr. Leon S. Stone of the Department of Anatomy; a case report, "Fatal uncomplicated measles," by Dr. George C. Wilson of the Department of Pathology; and a brief résumé of the outstanding features of the new educational program then being inaugurated at Yale. This paper, "The study of medicine at Yale," was contributed by the Chairman of the Committee on Curriculum, Dr. Raymond Hussey of the Department of Pathology.

The first item under Comments set forth the purpose behind the founding of the new publication and the hopes of the editors for its future:

> The natural and proper amalgamation of the Biological Sciences with Medicine which is obviously taking place in the scientific world is well demonstrated by the present interrelation and development of these branches at Yale. As a medium of expression of these departments at this University this JOURNAL has been inaugurated. . . . The addition of another publication to the already abundant field of scientific journalism has not been attempted without consideration and it is hoped that this JOURNAL will have certain characteristics which will prove its usefulness. In other words that it will represent something other than one more journal.
>
> Conceived by a small group of students in the Yale School of Medicine the JOURNAL is essentially a publication of and by the students in Medicine and the Biological

[3] 1935, *7*, 191-207.
[4] 1935, *8*, 113-126.
[5] 1937, *9*, 199-213.

Sciences in Yale University. In its pages it is purposed [*sic*] to present such subjects as will be of educational value and will further develop the concept of Biology and Medicine as it is envisaged by those working in these fields in this University. . . .

This section also included the announcement that Dr. Leon Stanfield Stone had been promoted to Professor of Anatomy and Dr. Allan King Poole to Assistant Professor of Medicine, and that three very able men had been brought to Yale from other places—Dr. Felix d'Herelle as Professor of Bacteriology in charge of the section of Proto-biology, Dr. John R. Paul as Assistant Professor of Medicine, and Dr. Daniel Cady Darrow as Assistant Professor of Pediatrics.

Another item of interest was the election to membership in Alpha Omega Alpha of five members of the fourth-year class: Walls Willard Bunnell, William Mason Hale, Vernon William Lippard, Philip Waddell Skinner, and Robert Tennant, Jr. The last pages of the section were devoted to a full description of three new buildings which had been added to the School of Medicine—an extension to Brady Memorial Laboratory, Lauder Hall, and Farnam Memorial Building, this being the era when great changes were being brought about under the leadership and prodding of Dr. Winternitz, when the curriculum was being drastically revised, the physical plant extensively enlarged, and the faculty strengthened and revivified.

The papers presented to the Yale Medical Society, which were abstracted by the student editors under Events, were five in number: "Studies in optic nystagmus" by James Charles Fox, Jr. and Raymond Dodge; "Electrolyte concentration and blood volume in infants with dehydration due to diarrhea and vomiting" by Daniel C. Darrow; "Pathogenesis of nephritic edema" by J. P. Peters, A. M. Wakeman, A. J. Eisenman, and Carter Lee; "A mechanism maintaining the hyperglycemia of diabetes" by H. E. Himwich, W. H. Chambers, Y. D. Koskoff, L. H. Nahum, and M. A. Adams; and "Some observations on ergothionine" by Blythe A. Eagles and Harry M. Vars.

The papers presented at the Clinical Congress were largely from men outside the School of Medicine—I. C. Rubin and Fred Wise of New York, Walter C. Alvarez and Leonard G. Rowntree from Rochester, Minnesota, David T. Smith of Ray Brook, New York, Robert B. Osgood of Boston, and Bertram L. Bryant of Bangor, Maine. These papers were all abstracted by the same student editor—the last paper receiving scant notice, for what reason we can only guess. It contained one sentence only and that not complete: "A discussion of the value of regular health examinations, together with suggestions as to how they may be conducted by the general practitioner."

Students likewise abstracted the articles published from the School of Medicine during 1927-1928, but members of the faculty contributed the four book reviews.

Many of the contributors of the other scientific papers which filled the pages of the first volume are familiar to present-day readers of the *Journal* since they still are to be found in its pages—Samuel C. Harvey, Francis G. Blake, Ira V. Hiscock, John P. Peters, Harold S. Burr, Hubert B. Vickery, Herman Yannet, Allan K. Poole, George R. Cowgill, William J. German, and C.-E. A. Winslow. Dr. J. Roswell Gallagher, who is at present Alumni Editor of the *Journal*, was the author of an article on "The colour vision of fish."[6] And a pattern was set in this first volume which was followed with considerable faithfulness in succeeding volumes—that of including an historical paper in almost every issue. Thus Dr. Harvey, in addition to an article on the treatment of wounds,[7] wrote on "The Education of Nathan Smith."[8] Dr. Russell H. Chittenden's address on "William Beaumont,"[9] presented at the dedication of the Beaumont Memorial Tablet on June 29, 1926 at Beaumont's birthplace in Lebanon, Connecticut, was included in the third issue. Another member of the first faculty of the School of Medicine (in addition to Nathan Smith) was the subject of a paper by Dr. Harold S. Burr—"Jonathan Knight and the founding of the Yale School of Medicine"[10] and in the same issue was another interesting historical paper by Theodore E. Zunder on "Joel Barlow and seasickness."[11]

Subsequent issues of Volume I announced the gift of $1,000,000 from the Rockefeller Foundation as endowment for the Yale School of Nursing which meant that this novel experiment in nursing education (its students were required to have a bachelor's degree for entrance), which had been under the leadership of Miss Annie W. Goodrich since its beginning in 1923, was permanently established. Announced also were gifts totalling $4,100,000 for the construction of the Raleigh Fitkin Memorial Pavilion, a new clinic building and medical and pediatric laboratory, and the Institute of Human Relations. The latter, along with the establishment of "The Human Welfare Group," represented, in the words of the editorial, "the results of eight years of careful thought, heart-break, and enthusiasm; results that are remolding medical education, directing the attention of thoughtful men into new channels, relighting the fires of high hopes for human progress."

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[6] 1929, *1*, 283-293.
[7] 1928, *1*, 79-83 (with Edward L. Howes).
[8] 1929, *1*, 259-268.
[9] 1929, *1*, 121-127.
[10] 1929, *1*, 327-343.
[11] 1929, *1*, 385-390. Joel Barlow, a member of the Class of 1778 of Yale College, who achieved fame as a political philosopher and as one of the "Hartford Wits," had described his thirty days of *mal de mer* crossing the Atlantic in a French packet in May of 1788 with all the detailed observation of a careful case report, but with considerably more color than is usually found in such reports. "The mouth of the Hudson," he wrote in his diary, "received the first sacrifice of the contents of my stomach, that of the Seine was honored with the last; & I hope that the palate of his Oceanic Godship was sufficiently sweetened with the copious libations of bile it received from my gallbladder, which I am sure was wrung to its very last drop for more than a thousand times."

"The human side of the news" included the intelligence that Courtney C. Bishop, Charles A. Breck, David Kalkstein, Marian Leonard, and Carl H. Wies had been elected to the Editorial Board of the *Journal;* that among those elected to the Yale Chapter of Sigma Xi were Dr. Philip B. Cowles (now Professor of Microbiology) and Dr. Vernon W. Lippard (now Dean of the Yale University School of Medicine); that Dr. John Farquhar Fulton, who had received his M.D. in 1927 from Harvard, had been appointed Professor of Physiology and Chairman of the Department and would join the faculty in 1930; and that the degree of Doctor of Medicine *cum laude* had been conferred upon Walls Willard Bunnell, Herman Yannet, and Vernon William Lippard, who had also won the Parker Prize.

The Yale Journal of Biology and Medicine, as was pointed out by Dr. Winternitz in the paragraph quoted from his Annual Report, was not the first medical journal at Yale. In November of 1894 there had appeared a forty-eight-page publication entitled *Yale Medical Journal,* which could be said to be the parent of the present *Journal.* Since there are a number of similarities between the two, a brief description of the *Yale Medical Journal* might be in order. The subscription price was two dollars a year; single copies, thirty cents. It was to be "Published monthly, from November to June, by Students of the Yale Medical School," there being six student editors and a faculty advisory board of five. A student chairman was elected each year to serve as editor and students ran the affairs of the *Journal* with far less help from the faculty, apparently, than is now given to the present publication.

The first issue announced that the purpose of the new venture was to offer students of medicine closer contact with the leaders of their profession and with each other and to provide a medium through which physicians, especially in Connecticut, could communicate with each other professionally. The first article in Volume I, Number 1, a "Preliminary report of an epidemic of paralytic disease, occurring in Vermont, in the summer of 1894," by C. S. Caverly, President of the Vermont State Board of Health, is now a classic. This article was the first published account of an epidemic of poliomyelitis in the United States and was considered to be of sufficient interest and importance to be re-issued in the *Connecticut State Medical Journal* in November 1943 with an introduction by Dr. Creighton Barker. Of particular note was the fact that thirteen (out of 123) cases had the non-paralytic form of the disease.

Other papers included "Sanitary examination of well water," "Mixed reflexes," "The increase of insanity," "The pathology of la grippe," and "Reflex neuroses due to eye-strain." This first issue also included an apology that because of pressure of work, William H. Carmalt, Professor of Surgery and Attending Surgeon to the New Haven Hospital, was unable to prepare the article on appendicitis which had been promised in the "Prospectus." Dr. Carmalt did, however, contribute a five-page article on

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FIG. 1. An "advertisement" of the Yale Medical School which appeared in the *Yale Medical Journal* (this one from Volume II). Following this there was a two-page advertisement of Yale College for which, according to a former editor, the *Journal* probably received no reimbursement.

the George Bronson Farnam Ward in the Hospital under "Hospital Notes" which became a regular feature of the *Journal*. In the Abstract section there appeared résumés of thirteen articles, largely from foreign journals. Another regular section, which created considerable interest among practitioners in the state, was that entitled Medical Society Reports. Herein accounts of the activities of various local societies found a continuing place and the *Journal* thus fulfilled its avowed function of keeping physicians informed about what was going on in medicine in Connecticut and nearby states. Alumni and School Notes comprised the final section of the publication.

Dr. Carmalt's paper on appendicitis did not appear in the December issue either, as had been hoped, but there were two papers, one on some common changes in the structure of the appendix vermiformis by Robert T. Morris of New York and the other on the etiology of appendicitis by Dr. M. Storrs, Surgeon to the Hartford Hospital. Interest in appendicitis was of course considerable at this time, Reginald Fitz's classic paper having appeared in 1886. Professor Carmalt made a contribution to issue 3, however—a graphic description of an operation performed on the kitchen table in a farmhouse in Hamden, the patient being a twelve-year-old boy who had five gunshot wounds of the intestine. Carmalt wrote modestly, "The case is of local interest only, being, so far as I can learn, the first instance in this vicinity of perforating gunshot wounds of the intestines successfully treated by modern aseptic methods." However, by Volume IV (1897-1898) a similar successful recovery was reported from the neighboring town of Bridgeport where a drunken farmhand had shot himself in the abdomen causing nineteen perforations in all.

In issue 3 of Volume I there was an account of the first public graduation exercises of the Connecticut Training School for Nurses on December 13, 1894, at which each graduate received a badge bearing the motto "Semper Paratus," and an interesting address on the history of the school (founded in 1873) was given by Mrs. Francis Bacon, the wife of Dr. Bacon, senior attending surgeon to the New Haven Hospital.

A comparison of the medical problems discussed in the early pages of the *Yale Medical Journal* as contrasted with those in the present *Yale Journal of Biology and Medicine* is, as with any medical journal, a lesson in scientific progress. Some of the diseases and conditions which were problems fifty years ago have now largely disappeared from the literature, while others, such as cancer, poliomyelitis, diabetes, and tuberculosis, are still of vital concern. In the first volume of the *Yale Medical Journal* there were seven papers on diphtheria, six on tuberculosis, and six on typhoid, two on snake bite, one on insanity, among, of course, many others. Appendicitis received a great deal of attention; epidemics of yellow fever, largely in the south, were reported regularly. The *Journal* also carried articles on the use of electricity in medicine and the new X-rays. There was a paper on

acromegaly with fifteen figures in Volume IV (1897-1898),[12] with an extensive bibliography of the subject in a later issue,[13] both by Dr. O. T. Osborne, Professor of Materia Medica and Therapeutics at Yale University. In an earlier volume there had been a report by Dr. S. P. Goodhart on "Three cases of brain tumor which the writer had observed in clinics in Berlin and Vienna."[14]

Statistics appearing in Hospital and Clinic Notes in one of these early issues indicated that there had been 15,618 consultations for the year ending October 31, 1897 at the New Haven Dispensary.[15] Thirty-three countries were represented in these patients as follows: United States, 1,570; Russia, 1,099; Ireland, 1,077; Germany, 244; Italy, 230; England, 85, etc. During a six-week period 36 operations were performed in the New Haven Hospital, four of them for appendicitis.

In subsequent years we find contributions by a number of men whose names are an imperishable part of the history of this period—S. Weir Mitchell, "The Physician,"[16] William Henry Welch, "The relation of Yale to medicine,"[17] Simon F. Flexner, "Bubonic plague, its nature, mode of spread, and clinical manifestations,"[18] William Osler, "On the educational value of the medical society,"[19] Henry M. Hurd, "The duty and responsibility of the university in medical education,"[20] William Stewart Halsted, "The training of a surgeon,"[21] Harvey Cushing, "Instruction in operative medicine,"[22] W. W. Keen, "The community of interest of the medical school and the public,"[23] Eugene L. Opie, "Opsonins and their relations to the diagnosis and treatment of bacterial infection,"[24] Frederick C. Shattuck, "The science and art of medicine in some of their aspects,"[25] and W. H. Howell, "The medical school as part of the university."[26] Two noteworthy contributions from the faculty of the School are those of Joseph Marshall Flint: "Concerning the efficiency of the positive pressure method in thoracic surgery with the description of a new apparatus"[27] and "Compensatory

[12] Pp. 1-10. Dr. Osborne referred a later case of acromegaly to Dr. Harvey Cushing and it is reported on pages 154-158 of Cushing's *The pituitary body and its disorders*. Philadelphia, J. B. Lippincott Company, 1912.
[13] Pp. 85-99.
[14] 1896, *2*, 312-318.
[15] 1897, *4*, 142-143.
[16] 1900, *7*, 54-58. A poem read before the Congress of Physicians and Surgeons, May 3, 1900.
[17] 1901, *8*, 127-158. A particularly valuable contribution to the history of medicine at Yale delivered at the 200th anniversary of Yale College.
[18] 1902, *8*, 293-310.
[19] 1903, *9*, 325-336. An address at the 100th anniversary of the New Haven Medical Association.
[20] 1903, *10*, 1-17.
[21] 1904, *11*, 1-23.
[22] 1906, *12*, 855-879.
[23] 1906, *13*, 1-15.
[24] 1907, *13*, 301-321.
[25] 1907, *14*, 1-11.
[26] 1909, *16*, 45-65.
[27] 1910, *16*, 295-323.

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FIG. 2. A page picked at random from the advertisements in the *Yale Medical Journal*. In some issues there were as many as thirty-six ads for everything from Brooks Bros. clothes to wheel chairs and Lithia water.

hypertrophy of the small intestine following resection of large portions of the jejunum and ileum."[28]

With Number 9 of Volume XVIII (May, 1912) the *Yale Medical Journal* was discontinued—for two reasons according to the editors: one, because there was an abundance of available current medical literature for all desiring it; secondly, "because of the belief that the value of the *Journal* in the limited field which it has attempted to cultivate is not sufficient to justify the effort necessary for its continued publication." Other reasons might also have been influential in the decision. Dr. Samuel Harvey has pointed out that the effect of raising the requirements for entrance into the School of Medicine in 1908 reached its maximum in 1912. The editorial announcing the end of the *Journal*, however, also carried a statement about advertising to the effect that when the Proceedings of the State Medical Society were first published in the *Journal* and it became to that extent an official organ of the profession of the state, the policies concerning advertisements were revised to conform to the standard of the *Journal of the American Medical Association*. This meant dropping about half of the advertising matter which had previously been carried, and although the editors disavowed their dependence on this income, it may very well have been of some consequence in their decision to abandon the *Yale Medical Journal* after its successful and useful existence of eighteen years.

In the interim between 1912 and 1928, when *The Yale Journal of Biology and Medicine* was established, laboratory research was changing the whole face of medicine in this country, and the large sums of money being given by philanthropists to further research were an encouragement to promising young men to seek opportunities in the laboratory. During the last part of the nineteenth and the first quarter of the twentieth century preclinical chairs in medical schools were becoming full-time posts and laboratory facilities were provided. "Full-time" medicine received great impetus during the early part of the century, and gradually America developed strong research centers which drew students to these shores as they had been drawn first to England, then to France, and then to Germany during the eighteenth and nineteenth centuries.

This change in emphasis was reflected in the subject matter of the new *Journal* at Yale. Over half of the articles now dealt with "pure" research and those papers which were concerned with clinical problems had become, with the changing style of the time, less informal and discursive and more the type of case reporting that is considered most effective today. The economy in words is a definite improvement, but much of the color and interest is lost in the sterilized style of modern medical writing—for instance, such a title as "Our New England stomachs: their trials and

[28] 1910, *16*, pp. 283-335 of the Proceedings of the Connecticut State Medical Society published in No. 6 of the *Journal*.

tribulations," which appeared in Volume III of the *Yale Medical Journal*, would never be countenanced today.

But although research was receiving an increasing amount of attention, neither its clinical applications nor the cultural aspects of medicine to be found in its history were neglected by the editors of the *Yale Journal*. The result was a well-rounded publication which was soon justly earning a reputation far beyond the environs of the Yale School of Medicine. Dean Winternitz in his Annual Report for the academic year 1930-1931 had this to say:

> In as much as Professor Smith of this department [immunology] is editor-in-chief of the *Journal of Biology and Medicine*, it is in place to mention here the outstanding success of the *Journal* during the three years it has been in existence. The *Journal* has proved to be of increasing value as a literary and informative organ, and it will depict the activities of the School of Medicine and, in an increasing degree, those of the biological sciences at Yale accurately and uniquely for the historian of the future. More than this, it is proving to be a valuable part of the educational procedure, and it is hoped that an endowment will ultimately become available for it as such. It should also become an elective curricula activity. Among the gifts to the School, none are more deeply appreciated than those of a few friends who have made it possible to meet the deficit of a few hundred dollars entailed through the Journal, and it is a privilege to express that gratitude here. The publication is so valuable that there is no fear funds will not be available from year to year to carry it on, but it seems unwise to allow the *Journal* to go on without official University recognition and support. Only too often have extracurricula activities of this kind proved themselves to be of greater value than some of the more extravagant and costly traditional procedures included in the legitimate operations of an institution. Just how valuable the *Journal* has proved as an educational organ in one direction is perhaps best expressed by a letter recently received by the editor from one of the student contributors. The letter follows:
>
> "Now that my paper on Tetanus has been accepted for publication in the Journal, I would like to make some comments on what its preparation has meant to me.
>
> "It would seem to me, at least, that the publication of a journal is a logical and necessary outcome of the School's education policy. By providing any student who wishes to do some independent work with a friendly audience and a judicious criticism, it should further immeasurably the educational aims of the School. In a more didactic institution, where an article as pretentious as mine coming from a student would be considered impudent, there is no need for a Journal; in our School I do not see how we could do without one.
>
> "Personally, I feel that the preparation of that article on tetanus was as educating an experience as I have had, and it certainly was one of the most enjoyable. The help that I received from the staff was invaluable, and the sifting of the literature and the drawing of conclusions was the most satisfactory discipline imaginable. The study of a subject such as tetanus leads one into all the medical sciences, and it is no exaggeration to say that I learned more pharmacology and physiology, for instance, from working up that paper than I ever did from the courses or textbooks in these subjects. The work on the paper brought most clearly to me the correlation between the preclinical sciences and clinical medicine, and I found some of the older papers in bacteriology very thrilling—Behring's first work with antitoxins, Metchnikoff's phagocytosis work, etc. They have given me a real insight into the achievements and prejudices of the science of bacteriology.

"Work on the paper brought me into contact with the inexhaustible reservoirs of knowledge—physiological and historical—of the mind of Dr. Fulton, and on this account alone, the writing of it would have been invaluable to me. His willingness to explain things to me at any time, and the delightful lucidity of his analysis have benefited the paper greatly and have profited me even more. The help afforded me by the staff of the Pediatrics Department was equally beyond praise, and I like to think of the paper as a sort of monument to my service on pediatrics. In speaking of Dr. Powers as a physician and teacher it is difficult to keep from expressing one's admiration in terms which that modest man would abhor; in writing that article I found one more approach to his stores of knowledge, and one which I hope I was able adequately to utilize.

"Finally, heartiest thanks for your own able, kindly, and constructive criticism, as well as for your most generous praise.

"I hope that I have made clear how adequately, it seems to me, the Journal focusses the opportunities which the School offers for self-education."

This reaction is representative, in general, of the feeling of those students who are elected to serve on the editorial board of the *Journal*. In assisting with the reviewing, editing, and proofreading of papers under the supervision of the Editor and the senior editorial board they have the opportunity of closer contact with members of the faculty than is often possible, and the enthusiasm and inspiration engendered by these contacts have a salutatory effect which often reaches beyond the students themselves. This was an aspect of the educational function of the *Journal* that Dr. Smith fostered in particular, and many students were grateful for the rare privilege of sitting down with him to edit a paper. From such sessions much more was gained, of course, than facility with a blue pencil or a knowledge of what constitutes good medical writing.

In addition to the time and energy which Dr. Smith gave to the student editors from year to year, he contributed materially to the financial well-being of the *Journal*. As is indicated in both of the quotations from Dr. Winternitz' reports, the publication was never flourishing, financially speaking, and since Dr. Smith was personally responsible for it until it was incorporated in 1941, he more than once made up deficits from his own pocket or solicited funds from those members of the faculty whom he knew were sympathetic friends of the *Journal* and its program. At the present time it has more nearly reached that mythical state of being "in the black" than at any other period in its history. With something under 1,300 subscribers (112 of whom are foreign), the *Journal*, because of the increased costs of printing, was still not in a prosperous condition. But a return to the policy of accepting advertising and an arrangement with the Yale Alumni in Medicine by which their membership fees would be increased to include a subscription to the *Journal* have greatly bolstered its financial status. Problems of monetary exchange are a handicap to securing more subscriptions among scientists abroad, but the number of requests for reprints attests to the fact that those copies which go to libraries are well

used and that the *Journal* has a wider audience across the sea than is evident from the subscription list.

Over the years the *Journal* has offered to its readers papers which in perspective have been outstanding contributions to knowledge in certain fields. Early in its history, Dr. Smith and his associates began the custom of dedicating certain issues to distinguished members of the faculty and in these special numbers are to be found papers of unusual caliber. The first of such numbers was that of March 1932 in honor of Lafayette B. Mendel, Professor of Physiological Chemistry at Yale from 1921-1935 (and in the department since 1892).[29] Similar numbers have paid tribute to Harvey Cushing,[30] Willard Burr Soper,[31] James Dowling Trask,[32] C.-E. A. Winslow,[33] Milton C. Winternitz,[34] Samuel C. Harvey,[35] Grover F. Powers,[36] and Francis G. Blake.[37]

In addition to the historical papers already mentioned, the following have a timeless quality: John E. Lane, "William H. Carmalt,"[38] John R. Paul, "Nathan Smith and typhoid fever,"[39] E. H. Jenkins, "Benjamin Gale,"[40] George Blumer, "Eli Ives—Practitioner, teacher and botanist,"[41] William Snow Miller, "Elisha Perkins and the metallic tractors,"[42] and Ernest Caulfield, "Early measles epidemics in America."[43]

The Beaumont Medical Club (a small group of Connecticut physicians interested in the history of medicine) has been responsible for a number of papers by eminent authors, their annual public lecture being customarily printed in the *Journal*. Among them are to be found Sir Joseph Needham, "Limiting factors in the advancement of science as observed in the history of embryology,"[44] Richard Shryock, "The advent of modern medicine in Philadelphia 1800-1850,"[45] Ralph H. Major, "Hippocrates and the Island of Cos,"[46] Gregory Zilboorg, "Humanism in medicine and psychiatry,"[47] John E. Donley, "Harvey, Riolan and the discovery of the blood,"[48]

[29] 1932, *4*.
[30] 1939, *11*. This volume was notable for the fact that its contributors were of a younger generation, they being directly or indirectly pupils of Cushing.
[31] 1943, *15*. This issue was devoted to tuberculosis.
[32] 1944, *16*.
[33] 1947, *18*. The forty papers in this number testify to the breadth of Dr. Winslow's interests.
[34] 1950, *22*.
[35] 1951, *23*.
[36] 1952, *24*.
[37] 1952, *24*.
[38] 1929, *2*, 89-113.
[39] 1930, *2*, 169-181.
[40] 1930, *2*, 257-268.
[41] 1932, *4*, 649-663.
[42] 1935, *8*, 41-57.
[43] 1943, *15*, 531-556. A classic contribution to the history of measles.
[44] 1935, *8*, 1-18.
[45] 1941, *13*, 715-738.
[46] 1941, *14*, 1-11.
[47] 1944, *16*, 217-230.
[48] 1946, *18*, 319-331.

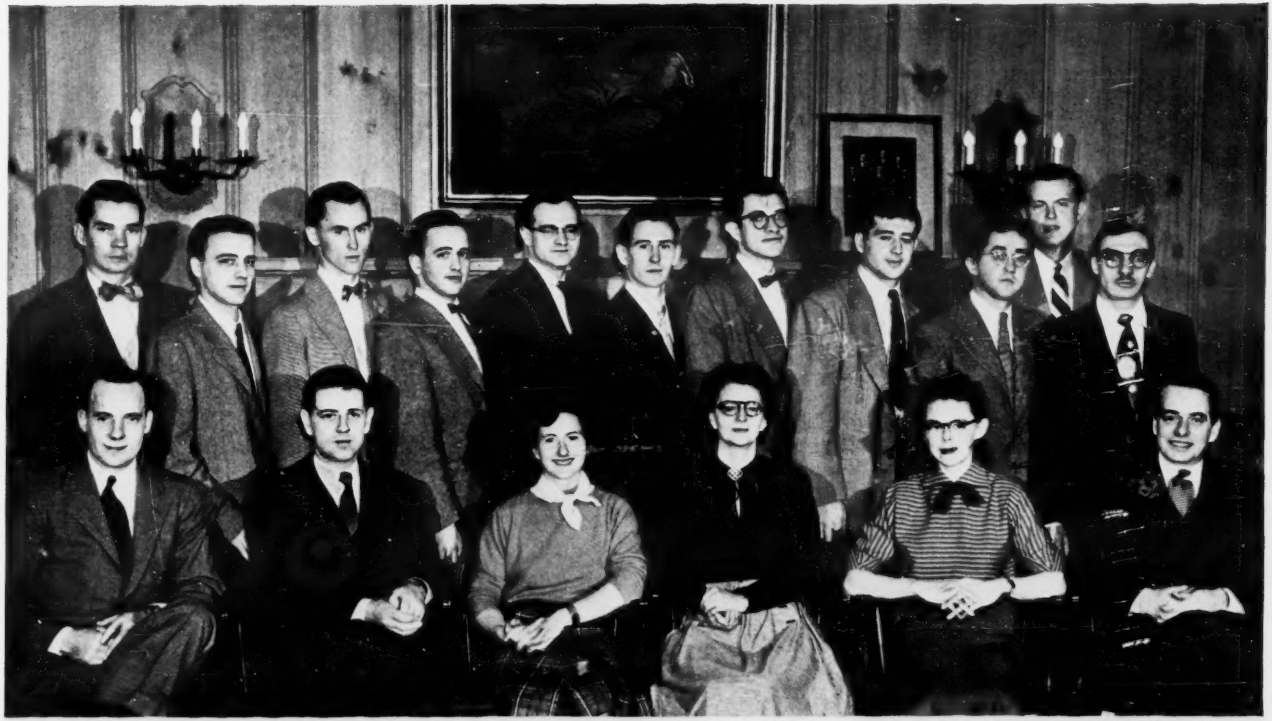
Chauncey D. Leake, "Ashbel Smith M.D. 1805-1886: Pioneer educator in Texas,"[49] Josep Trueta, "Michael Servetus and the discovery of the lesser circulation,"[50] and Peter Pineo Chase, "The ailments and physicians of Dr. Johnson."[51]

An especially interesting historical number was that of January 1934 (Volume VI) which marked the 150th anniversary of the New Haven Medical Society, one of the oldest in the country. In addition to a facsimile of *Cases and observations by the Medical Society of New-Haven County, in the State of Connecticut, instituted in the year 1784,* originally published in 1788, there appeared papers by George Blumer,[52] Herbert Thoms,[53] Creighton Barker,[54] Harold S. Burr,[55] Thomas H. Russell,[56] and Henry E. Sigerist.[57]

Of the many excellent scientific papers only a few can be mentioned. The following, listed chronologically, seem worthy of notice.

Author	*Title*	*Reference*
John P. Peters	Acid-base equilibrium and salt and water exchange	1930, *2*, 183–221
David Kalkstein	The millimol and milli-equivalent	1930, *2*, 351–361
John Paul, James D. Trask, and R. Salinger	Comparative statistical analyses of the 1916 and 1931 epidemics of poliomyelitis in and about the city of New Haven	1932, *5*, 39–54
John P. Peters	The distribution and movement of water and water solutes in the human body	1932, *5*, 431–467
Eugene M. Landis	Factors controlling the movement of fluid through the human capillary wall	1932, *5*, 201–225
Hugh Cairns	The ultimate results of operation for intracranial tumours: A study of a series of cases after a nine-year interval	1936, *8*, 421–492
Herbert Thoms and Hugh M. Wilson	Lateral roentgenometry of the pelvis — a newly modified technic	1937, *9*, 305–309
John H. Lawrence	Artificial radio-activity and neutron rays in biology and medicine	1937, *9*, 429–435

[49] 1948, *20*, 225-232.
[50] 1948, *21*, 1-15.
[51] 1951, *23*, 370-379.
[52] The first medical transactions in America, pp. 299-305.
[53] The beginnings of medical practice in New Haven Colony, pp. 307-321.
[54] The founding of the New Haven County Medical Association, pp. 323-331.
[55] The founding of the Medical Institution of Yale College, pp. 333-340.
[56] Events in the Association after 1840, pp. 341-350.
[57] Medical societies, past and present, pp. 351-362.



C. T. Alburtus

FIG. 4. Members of the editorial board of *The Yale Journal of Biology and Medicine* in February 1952. Standing, left to right: John F. Snyder, Jack Peter Green, Wilbur P. McNulty, Jr., Nicholas A. Halasz, Kenneth G. Bartels, Robert R. L. Guillard, Jacques M. Quen, Arthur C. Crovatto, Raymond Rappaport, Jr., John Keith Rose, and Ralph Gross. Sitting: William F. Stephenson, John R. Brobeck, Ora L. Kingsley, Elizabeth H. Thomson, Virginia S. Green, and Frederick G. Kilgour.

FIG. 3. The student editors in 1910. Standing, left to right: Frank Elmer Johnson, Samuel Clark Harvey, Louis Henry Levy. Sitting: William Henry Beardsley, Herbert King Thoms, *Chairman*, Harry St. Clair Reynolds. Dr. Harvey was Chairman in his fourth year (1912).

Author	*Title*	*Reference*
Alfred J. Vignec, John R. Paul, and James D. Trask	The recovery of the virus of poliomyelitis from extra-neural sources in man, with a survey of the literature	1938, *11*, 16–31
Herbert Thoms and Milton S. Godfried	The interrelationships between fetal weight, size of pelvic inlet, and maternal height	1939, *11*, 355–362
Grover F. Powers	Developments in pediatrics in the past quarter century	1939, *12*, 1–22
H. M. Zimmerman	The pathology of the nervous system in vitamin deficiencies	1939, *12*, 23–28
W. U. Gardner and E. Allen	Malignant and non-malignant uterine and vaginal lesions in mice receiving estrogens and estrogens and antigens simultaneously	1939, *12*, 213–234
Joseph F. Sadusk, Jr., Francis G. Blake, and Anne Seymour	Observations on the absorption, excretion, diffusion, and acetylation of sulfathiazole in man	1940, *12*, 681–696
Carroll A. Pfeiffer, Arthur Kirschbaum, and William U. Gardner	Relation of estrogen to ossification and the levels of serum calcium and lipoid in the English sparrow, *Passer domesticus*	1940, *13*, 279–284
L. S. Stone and C. H. Cole	Grafted eyes of young and old adult salamanders (*Amblystoma punctatum*) showing return of vision	1943, *15*, 735–754
John R. Brobeck, Jay Tepperman, and C. N. H. Long	Experimental hypothalamic hyperphagia in the albino rat	1943, *15*, 832–853
Jay Tepperman, John R. Brobeck, and C. N. H. Long	The effects of hypothalamic hyperphagia and of alterations in feeding habits on the metabolism of the albino rat	1943, *15*, 855–874
George Sayers, Marion A. Sayers, Edith G. Fry, Abraham White, and C. N. H. Long	The effect of the adrenotropic hormone of the anterior pituitary on the cholesterol content of the adrenals. With a review of the literature on adrenal cholesterol	1944, *16*, 361–392
Leonell C. Strong	Genetic analysis of the induction of tumors by methylcholanthrene. XII. The effects of selection toward resistance	1946, *17*, 145–155
Sir Howard Florey	The use of micro-organisms for therapeutic purposes	1946, *19*, 101–117

Author	*Title*	*Reference*
Horace T. Gardner and John R. Paul	Infectious mononucleosis at the New Haven Hospital 1921-1946	1947, *19*, 839–853
Joseph S. Fruton	The isolation of pure amino acids	1947, *19*, 999–1012
José M. R. Delgado	Permanent implantation of multilead electrodes in the brain	1952, *24*, 351–358
E. Grunberg and R. J. Schwitzer	The effect of hydrazine derivatives of isonicotinic acid on the infection of mice with M. Tuberculosis var. Bovis	1952, *24*, 359–365

The Yale Journal of Biology and Medicine goes forward under Dr. Robert H. Green, Associate Professor of Medicine, as the new Editor. Although illness forced Dr. Smith to give up the helm in 1949, he has been a sympathetic friend in the background. The news of his death* has brought sadness to all those who have been associated with him over the years, but the traditions which he has given to the *Journal* ensure that those who carry on will continue to make it a living tribute to his name.

ACKNOWLEDGMENT

Grateful acknowledgment is made to Dr. Samuel C. Harvey for his assistance, graciously offered and freely given, in the assembling of these notes.

* July 7, 1952.

PROTEIN THROMBOKINASE AND LIPOID THROMBOPLASTIN AS DISTINCT FACTORS WITH COMPLEMENTARY FUNCTIONS*

J. H. MILSTONE

Two classes of biologic agents can accelerate the production of thrombin from partially purified prothrombin. One class is well represented by platelets, the other by a factor obtainable from the plasma globulins. Representatives of both classes are found not only in blood, but also in crude tissue extracts. These two classes of agents are distinct; and they serve different functions in the clotting process.

The two groups of factors as studied in this laboratory[15, 18] correspond essentially to two old factors; and these factors will be called by their old, familiar names.

When Morawitz[19] introduced the term "thrombokinase" in 1904, he likened the activation of prothrombin by thrombokinase to the activation of trypsinogen by enterokinase. Pursuing this analogy, he considered the possibility that thrombokinase, like enterokinase, was an enzyme. Moreover, he explicitly recognized that thrombokinase was available in ordinary oxalated plasma.[20] Several further inferences made by Morawitz may or may not have been correct; nevertheless, his brilliant theory cannot be ignored. And it would be hard to deny that a basic property for defining thrombokinase, as conceived by him and as construed by later authors, was its capacity to activate prothrombin.

When Nolf[15] introduced the term "thromboplastic substances" in 1908, he considered these substances to be "only adjuvants." He did not propose that they activated prothrombin. In 1911-1912, Howell[10, 11] specifically denied that thromboplastin was a direct activator of prothrombin; and he further stated ". . . thrombokinase in the sense used by Morawitz does not exist. . . ." To Howell, thromboplastin was a factor found in preparations of brain cephalin and in platelets; and it functioned by "neutralizing" antithrombin. In 1935, Howell[12] reiterated these views, but identified the anticoagulant as heparin rather than antithrombin. After thromboplastin has disposed of heparin, "prothrombin is activated to thrombin by calcium without the coöperation of an organic kinase."

Thrombokinase and thromboplastin, originally conceived so differently, have sometimes been treated as if they were identical. There have been many reasons. The problem has long been obscured by a haze of unproven assumptions. Perhaps the most misleading of these has been the notion that

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one may define a factor simultaneously as "the tissue factor" and as "the prothrombin activator," without first proving that "the tissue factor" *is* "the prothrombin activator" and without appreciating that at least two different factors can be obtained from crude tissue extracts. If clotting factors were defined primarily in terms of their biochemical function, without reference to the unsettled "tissue factor" problem, thrombokinase could not be confused with Howell's thromboplastin.

Because this is the crucial point in need of clarification, it seems desirable to give a detailed report of experiments which reveal several contrasts between the two agents. The results show that protein thrombokinase and lipoid thromboplastin are distinct factors, with different functions.

Materials and Methods

Buffer. 0.9 per cent NaCl; 0.02M veronal, pH 7.4,[15] ionic strength 0.174.

Ca. 25 ml. 0.11M $CaCl_2$ plus 75 ml. buffer.

0.057 Oxalate. 0.057M $K_2C_2O_4$, ionic strength 0.17.

1.0M Oxalate: 0.1M Phosphate.

$K_2C_2O_4 + H_2O$	18.40	Gm.
K_2HPO_4 (Anhydrous)	1.74	Gm.
HCl 0.1N	15.0	ml.
Distilled Water to	100.0	ml.

Ionic strength 3.285, pH 7.34 (glass electrode).

Prothrombin (Prothrombin preparation III). The procedure for prothrombin preparation II was followed, up through the precipitation at pH 4.4.[15] Then the precipitate (instead of the supernatant as for preparation II) was taken as the prothrombin reagent. It was suspended in 10 ml. distilled water and stored at —17° C. For each day's experiments, the suspension was thawed and a fresh solution was made by diluting a small portion with 9 volumes of buffer.

Kinase. Concentrated thrombokinase, prepared from plasma globulin.[16]

Thrombin. The thrombin used was prepared as previously described.[15]

Crude globulin. Crude plasma globulin, after removal of fibrinogen by heating at 51° C.[16]

Lipoid thromboplastin (Lipid). That fraction of bovine brain, soluble in ether but not in acetone; 10 per cent suspension.[17] A similar preparation, containing both cephalin and lecithin, was used by Eagle.[4] Ferguson's[8] studies showed that clotting activity occurred in several of Folch's subfractions of crude brain cephalin. The possibility that true cephalin is a potent thromboplastin was left open. In a study of lung phosphatides, Cohen and Chargaff[1] reported that the "lecithin" and "cephalin" fractions showed approximately equal clotting activity. Leathes and Mellanby[13] reported that brain lecithin, but not brain cephalin, potentiated thrombokinase. Such considerations made it desirable to use a rather complete lipoid thromboplastin for the present work.

Trypsin. Dialyzed stock solution of crystallized trypsin.[17]

The methods were those described previously.[16] When prothrombin was activated in the presence of ionic calcium, the relative thrombin values were derived by dividing 3,000 by the observed clotting time. But for activation in the presence of oxalate it was necessary to use a set of empirical curves; for the speed of clotting was not precisely proportional to the amount of thrombin under these conditions. With concentrated thrombokinase, the activation of prothrombin could not be expected to stop upon admixture with oxalated fibrinogen, although it would be retarded by the usual fourfold dilution. The error is slight when the clotting times are short in comparison with the activation times.

The same prothrombin suspension at the same dilution, the same stock of thrombokinase, and the same stock of lipoid thromboplastin were used throughout this work. The activation mixtures contained prothrombin in the dilute state—about one-sixth of the prothrombin concentration of normal plasma. Prolonged activation of this diluted prothrombin under a variety of conditions did not yield significantly more thrombin than that indicated by the tables. The experiments of any one table were run in parallel, at the same time; and within any one table, the experiments are closely comparable on a quantitative basis.

The Complementary Action of Thrombokinase and Lipoid Thromboplastin

Figure 1 illustrates an experiment in which the same amounts of prothrombin and calcium were included in all tests. In the test with both kinase and lipid, thrombin was produced much faster than with twice as much kinase without lipid, or with twice as much lipid without kinase. The extra portion of kinase did not substitute for the missing lipid, nor did the extra portion of lipid substitute for the missing kinase. It is clear that kinase and lipid have different effects and that they complement each other.

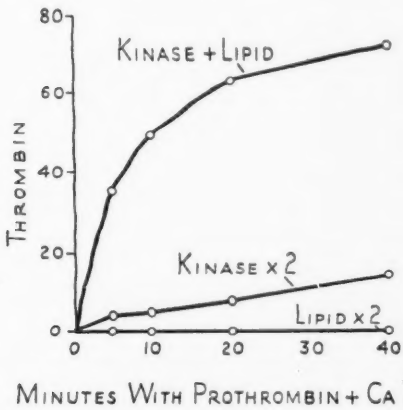


FIG. 1. Test system: prothrombin and calcium. With added thrombokinase and lipoid thromboplastin together, thrombin was produced much faster than with twice as much kinase or twice as much lipid. The activation mixture represented by the top curve had kinase diluted 1/176 and lipid diluted 1/880.

Control experiments showed that kinase plus lipid plus calcium did not produce thrombin.

Figure 1 also shows that kinase had some effect without added lipid. But without kinase, lipid plus calcium did not activate the prothrombin at all. This suggests that kinase is the primary, direct activator of prothrombin, whereas the lipid exerts an accessory effect provided that kinase is also present.

The experiment shown in Figure 1 did not quite eliminate the possibility that the lipid only magnified the effect of the thrombin without actually causing its faster production. In the experiment reported in Table 1, the lipid did not make the thrombin clot fibrinogen faster, i.e., it did not magnify the effect of the thrombin. Finally, it must be remembered that the amount of thrombin found at any one time is a net value—the total amount of thrombin produced, minus that which has been lost through inactivation or side reactions. It is concluded that the lipid accelerated the net production

of thrombin, and that this was not a spurious effect attributable merely to a shift in the thrombin assays.

In brief, thrombokinase activated prothrombin in the presence of calcium ions, whereas lipoid thromboplastin did not. Lipoid thromboplastin accelerated production of thrombin when the system contained not only prothrombin, but also calcium ions and thrombokinase.

TABLE 1

A TEST FOR THE EFFECT OF LIPOID THROMBOPLASTIN ON ASSAYS FOR THROMBIN

Mixtures				*Time after mixing*		
0.8 ml.	0.1 ml.	0.1 ml.	0.1 ml.	10 min.	20 min.	40 min.
				sec.	sec.	sec.
Buffer	Thrombin	Buffer	Ca	79	78	82
"	"	Lipid 1/20	"	85	83	82

Figures in the body of the table give clotting times of 0.1 ml. samples mixed with 0.3 ml. oxalated fibrinogen.

Kinase vs. Lipid with regard to Calcium and Oxalate

Some clotting effects are augmented by the addition of calcium ions; and some of these effects are strongly opposed by oxalate or citrate. The present experiments deal with such easily demonstrable effects. Whether some of these effects involve an equilibrium with organically bound calcium, and whether tightly bound calcium is important even in the presence of oxalate, are not of immediate concern. It is first important to know which effects obviously require ionic calcium, and which reactions can proceed in the presence of oxalate.

TABLE 2

ACTIVATION OF PROTHROMBIN BY VARYING AMOUNTS OF KINASE, IN THE PRESENCE OF 0.01 M OXALATE

Activation mixtures				*Period of activation*				
0.7 ml.	0.1 ml.	0.2 ml.	0.1 ml.	10 min.	20 min.	40 min.	80 min.	160 min.
				sec.	sec.	sec.	sec.	sec.
Buffer	Prothrombin	Oxalate	Kinase undil.	61	37	24	18	14
"	"	"	" 1/2	111	57	35	23	16
"	"	"	" 1/4	224	118	61	37	23
"	"	"	" 1/8	480	240	136	66	37
"	"	"	" 1/16	1040	535	285	155	71
"	"	"	" 1/32	2040	1380	655	340	152
"	Buffer	"	Kinase undil.	1500	1380	1500	...	1380

The stock solution of potassium oxalate was 0.057 M. Serial two-fold dilutions of kinase in buffer. Figures in body of table give clotting times of 0.1 ml. samples mixed with 0.3 ml. fibrinogen.

The activation of dialyzed prothrombin by dialyzed thrombokinase proceeded in the presence of 0.01 M oxalate, as shown in Table 2. Table 3 shows that the concentration of oxalate could be varied over a fourfold range without affecting the rate of activation. In all these tests, the ionic

TABLE 3

ACTIVATION OF PROTHROMBIN BY A CONSTANT AMOUNT OF KINASE IN THE PRESENCE OF VARYING CONCENTRATIONS OF OXALATE

Activation mixtures				*Period of activation*			
Buffer	Prothrombin	Oxalate	Kinase	10 min.	20 min.	40 min.	80 min.
ml.	ml.	ml.	ml.	sec.	sec.	sec.	sec.
0.8	0.1	0.1	0.1	135	84	55	43
0.7	"	0.2	"	134	92	51	40
0.5	"	0.4	"	133	81	50	39

The stock solution of potassium oxalate was 0.057 M, having an ionic strength of 0.171. The ionic strength of the buffer was 0.174. Serial two-fold dilutions of kinase in buffer. Figures in body of table give clotting times of 0.1 ml. samples mixed with 0.3 ml. oxalated fibrinogen.

TABLE 4

ACTION OF KINASE IN THE PRESENCE OF 0.5 M OXALATE

Activation mixtures				*Period of activation*					
Oxalate	Prothrombin	Buffer	Kinase	10 min.	20 min.	40 min.	80 min.	140 min.	160 min.
ml.	ml.	ml.	ml.	sec.	sec.	sec.	sec.	sec.	sec.
0.5	0.1	0.3	0.1	220	115	100	105		70
"	0.1	0.4	0.0	n.c.	n.c.			n.c.	
"	0.0	0.4	0.1	2220	2220				

For these tests 1.0 M oxalate: 0.1 M phosphate was used, making the activation mixture 0.5 M with respect to oxalate and 0.05 M with respect to phosphate. The ionic strength of the activation mixtures was 1.73. n.c. = no clot in 1 hour. Figures in the body of the table give clotting times of 0.1 ml. samples mixed with 0.3 ml. oxalated fibrinogen.

strength was practically constant, within the range 0.170-0.174. However, when the concentration of oxalate was raised to 0.5 M for the experiment shown in Table 4, the ionic strength became ten times as great, 1.73. Nevertheless, the kinase still activated prothrombin. The second test in Table 4 shows that the high ionic strength did not cause activation of the prothrombin in the absence of kinase. The last test, like the last in Table 2, reveals that the kinase preparation alone caused very slow coagulation of the

fibrinogen, and that this effect was too small to interfere with the tests. It may be noted that 0.5 M oxalate is 25-50 times as much as is ordinarily used to prevent coagulation of whole blood.

Figure 2 shows that the early rate of thrombin production, in the presence of 0.01 M oxalate, was directly proportional to the amount of added kinase. This is consistent with the hypothesis that thrombokinase is an enzyme.

Although the reagents were dialyzed, it may still be suspected that 0.01 M oxalate was not really an excess. Therefore, it should be reported that all the oxalated mixtures of Table 2 and Figure 2 remained clear until the end of the experiment. Then a small amount of calcium chloride was added; and all mixtures formed a dense white cloud. Thus, during the experiment, there had been enough oxalate to produce a heavy precipitate, but there had been a lack of ionic calcium.

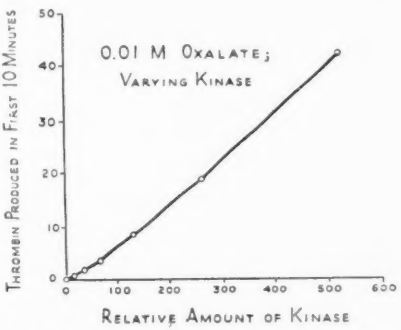


Fig. 2. Test system: prothrombin and oxalate. The early rate of thrombin production was directly proportional to the amount of added thrombokinase.

It may be suspected that the experiments did not allow enough time for the oxalate to bind the calcium. But the time allowed was much longer than that in which oxalate must act on whole blood to prevent the usual 5-10-minute coagulation. Moreover, it has been shown previously[18] that kinase can activate prothrombin continuously for seven hours at room temperature in the presence of excess oxalate.

Why, then, is ionic calcium ordinarily so important for the production of thrombin? Figure 1 shows that when the kinase is dilute and the test reagents partially purified, lipoid thromboplastin greatly accelerates the net rate of thrombin production. As has been reported elsewhere, the accessory thromboplastins so far studied do not exert this effect unless ionic calcium is present. This applies alike to brain lipid, to platelets, and to the accessory thromboplastin found in the globulin fraction of slaughter-house plasma.[18] It is likely that accessory thromboplastic action plays a very important rôle in the clotting of whole blood. For this rôle, ionic calcium is needed.

With the present preparations of prothrombin and kinase, the system becomes very sensitive to the addition or omission of calcium ions whenever an accessory thromboplastin is also included. When there is no difference,

with or without calcium ions, this is taken as an indication that the system is free of accessory thromboplastins of the type so far studied. Such a conclusion is valid only when the kinase-prothrombin system is known to respond to added thromboplastin plus calcium.

Table 5 shows that the addition or omission of calcium chloride made very little difference in the activation of prothrombin by kinase. At the end of a half-hour, the mixtures were adjusted to have the same calcium concentration for the thrombin tests; 30 seconds later, the critical thrombin assays were performed. Within the limits of error, as much thrombin had been produced in 30 minutes without added calcium plus ½ minute with calcium, as had been produced by the control mixture in 30½ minutes with calcium.

TABLE 5

SHOWING THAT CALCIUM CHLORIDE HAS LITTLE EFFECT ON THE ACTIVATION OF PROTHROMBIN BY THE KINASE REAGENT

Activation mixtures				*Incubated*	*Added*	*Then incubated*			
0.7 ml.	0.1 ml.	0.1 ml.	0.1 ml.	30 min.	0.1 ml.	½ min.	5 min.	10 min.	20 min.
						sec.	sec.	sec.	sec.
Buffer	Prothrombin	Ca	Kinase		Buffer	42	42	38	34
"	"	Buffer	"		Ca	46	42	37	31

Figures in the body of the table give clotting times of 0.1 ml. samples mixed with 0.3 ml. fibrinogen. No samples were taken during the first 30-minute incubation period.

The subsequent thrombin assays were performed to show that the mixtures were not completely activated at the time of the critical tests, and hence the critical tests measured the amount of thrombin produced in a definitely known time. Since the rate of thrombin production was not materially influenced by the addition of calcium ions, the presumption is that neither the prothrombin nor kinase contained enough accessory thromboplastin to influence the present tests.

The foregoing experiment was arranged so that no correction was required for the effect of calcium on the thrombin assays. However, previous experiments[18] have shown that even when this consideration was deliberately neglected and no correction made, the difference, with and without calcium, was not great.

In summary, lipoid thromboplastin and ionic calcium were neither absolutely necessary (Fig. 2, Tables 2-5) nor together sufficient (Fig. 1) for the activation of prothrombin. Thrombokinase was necessary (Fig. 1) and sufficient (Fig. 2). Although lipoid thromboplastin did not perform its accessory function without ionic calcium,[18] thrombokinase activated prothrombin in the presence of excess oxalate.

Quantitative differences

In the experiments of Tables 6 and 7, the concentrations of prothrombin and calcium were constant throughout. In Table 6, the tests had a constant amount of lipid, but varying amounts of kinase. With the four largest amounts of kinase, activation was too fast to permit estimation of the early rate from the five- or ten-minute thrombin assays. For the remaining six tests, covering a 32-fold range of variation in concentration of kinase, the amount of thrombin produced in ten minutes was directly proportional to

TABLE 6

ACTIVATION OF PROTHROMBIN BY VARYING AMOUNTS OF KINASE WITH CONSTANT AMOUNT OF BRAIN LIPID

Activation mixtures						*Period of activation*			
0.7 ml.	0.1 ml.	0.1 ml.	0.1 ml.	0.1		5 min.	10 min.	20 min.	40 min.
						sec.	sec.	sec.	sec.
Buffer	Prothrombin	Lipid 1/20	Ca.	Kinase	undil.	31	22	17	
"	"	"	"	"	1/2	33	23	18	
"	"	"	"	"	1/4	39	23	20	
"	"	"	"	"	1/8	48	32	23	
"	"	"	"	"	1/16	64	42	31	
"	"	"	"	"	1/32		77	45	33
"	"	"	"	"	1/64		128	77	51
"	"	"	"	"	1/128		310	177	106
"	"	"	"	"	1/256		1020	505	263
"	"	"	"	"	1/512		3600	1980	780
"	"	"	"	Buffer			>3600	>1800	1200

Serial two-fold dilutions of kinase in buffer. Calcium was added 30 seconds before kinase; and activation was timed from the addition of kinase. Figures in the body of the table give clotting times of 0.1 ml. samples mixed with 0.3 ml. oxalated fibrinogen.

the amount of kinase. This linear relation is illustrated graphically in Figure 3a. The largest amount of kinase plotted in Figure 3a is $\frac{1}{16}$ as great as the largest amount in Figure 2. This is, in part, a reflection of the fact that the present preparation of kinase is much more effective in the presence of lipid and calcium.

The last test of Table 6 shows that some thrombin was produced, very slowly, when no kinase was added. This is attributed to the presence of a small amount of kinase in the prothrombin reagent. Similar tests have been used in this laboratory to estimate the varying magnitude of such contamination in different prothrombin preparations.

Table 7 shows tests with constant kinase and varying lipid. Great variations in the amount of added lipid made little difference in the amount

TABLE 7

PRODUCTION OF THROMBIN IN THE PRESENCE OF VARYING AMOUNTS OF BRAIN LIPID WITH CONSTANT AMOUNT OF KINASE

Activation mixtures				*Period of activation*					
0.7 ml.	0.1 ml.	0.1 ml.	0.2 ml.	10 min.	20 min.	40 min.	80 min.	120 min.	420 min.
				sec.	sec.	sec.	sec.	sec.	sec.
Buffer	Prothrombin	Lipid 1/10	Kinase + Ca.	64	37	29	24	22	19
"	"	" 1/20	"	56	35	30	24	23	19
"	"	" 1/40	"	53	39	27	25	23	20
"	"	" 1/80	"	56	40	30	27	24	21
"	"	" 1/160	"	64	45	38	30	28	21
"	"	" 1/320	"	80	55	41	32	28	21
"	"	" 1/640	"	108	67	43	38	30	21
"	"	" 1/1280	"	145	90	64	47	34	23
"	"	" 1/2560	"	210	138	82	48	42	22
"	"	Buffer	"	560	280	152	78	56	22

"Kinase + Ca," added as a single reagent, was a mixture of equal parts of 0.0275 M Ca and a $\frac{1}{8}$ dilution of kinase. Figures in body of table give clotting times of 0.1 ml. samples mixed with 0.3 ml. oxalated fibrinogen.

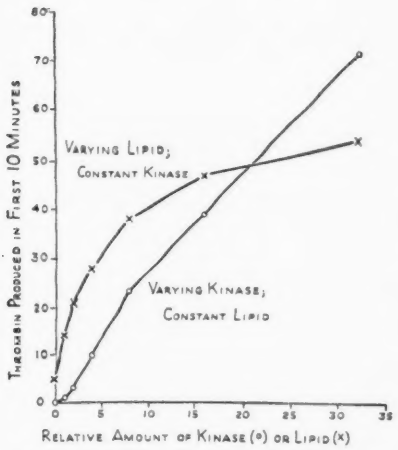


FIG. 3a. Test system: prothrombin, calcium, thrombokinase, and lipoid thromboplastin. The early rate of thrombin production was directly proportional to the amount of kinase, but not to the amount of lipid.

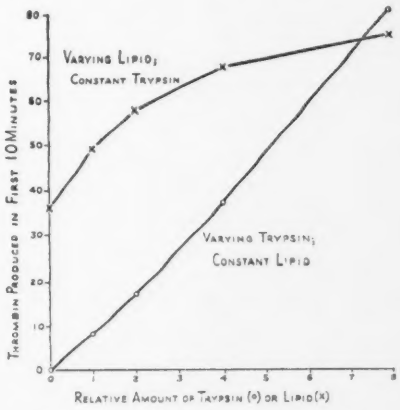


FIG. 3b. Test system: prothrombin, calcium, trypsin, and lipoid thromboplastin. The early rate of thrombin production was directly proportional to the amount of trypsin, but not to the amount of lipid.

of thrombin ultimately produced. The rate of thrombin production was determined by, but was not proportional to, the amount of added lipid. This relationship, for the last seven tests, is illustrated graphically in Figure 3a, where it offers a sharp contrast to the "varying kinase" line.

The first three tests in Table 7 show that there was an optimal amount of lipid. Further increase beyond this led to a slightly slower rate of thrombin production. The other end of the scale is particularly interesting, because a sharp acceleration was caused by lipid in a dilution of 1/2560 x 1/11, corresponding to approximately 3.6 micrograms per ml. of activation mixture.

Similar patterns of results were obtained when crystallized pancreatic trypsin was substituted for thrombokinase, as illustrated in Figure 3b. The

TABLE 8

HEAT LABILITY OF KINASE

Activation mixtures					*Period of activation*			
0.7 ml.	0.1 ml.	0.1 ml.	0.1 ml.	0.1 ml.	10 min.	20 min.	40 min.	80 min.
					sec.	sec.	sec.	sec.
Buffer	Prothrombin	Oxalate	Oxalate	Kinase	134	92	51	40
"	"	"	"	Heated kinase	n.c.	3600	1800	840
"	"	Lipid 1/20	Ca.	Kinase	32	22	18	15
"	"	"	"	Heated kinase	600	300	157	91
"	"	"	"	Buffer	n.c.	n.c.	2400	1020

Kinase heated in water-bath at 60° C. for 15 minutes. The stock oxalate was 0.057 M. n.c. = no clot in one hour.

chief difference was that, without added lipid, trypsin had more effect than the kinase preparation. The "varying lipid" curve covered the range of the four smallest amounts used in the experiment of Table 7. The concentration of trypsin ranged between 0.068 and 0.544 micrograms per ml. of prothrombin activation mixture. In this range, the early rate of thrombin production was directly proportional to the amount of trypsin.

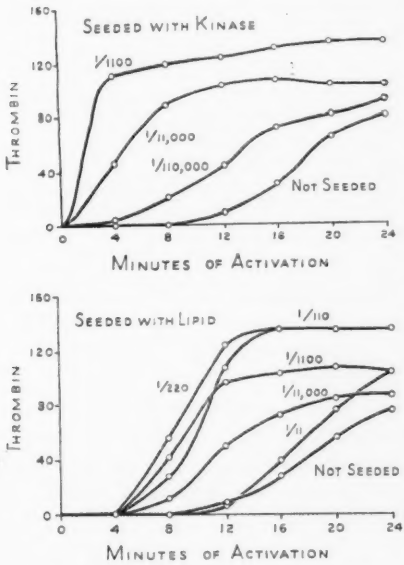
Trypsin could also substitute for thrombokinase in that trypsin activates prothrombin in the presence of oxalate. All this is in harmony with the hypothesis that thrombokinase is an enzyme. (Cf. Eagle and Harris.[5])

To what extent the "varying lipid" lines are curved because of the mechanism of the lipid effect, and to what extent the curvature might be due to an anticoagulant contaminating the lipid, must be left for future study.

Disregarding theoretical details, the experimental facts can be summarized simply. With the present materials, the early rate of thrombin production was directly proportional to the amount of thrombokinase; it was not directly proportional to the amount of lipoid thromboplastin.

Heat lability

Morawitz[19] pointed out that his thrombokinase was heat-labile. Table 8 shows that thrombokinase derived from slaughter-house plasma is heat-labile. More than 90 per cent of the activity was lost within 15 minutes at 60° C. Nevertheless, some activity could still be detected in the heated kinase, when tested either in the presence of oxalate or in the presence of lipid and calcium.



FIGS. 4a and 4b. Test system: crude globulin and calcium. Seeding with thrombokinase obliterated the latent period; seeding with lipoid thromboplastin did not. Figures on the chart give the final dilutions of the seed materials in the activation mixtures.

Howell[11] reported: "The aqueous solution of the phosphatid may be boiled without injury to its thromboplastic activity." It was found that the lipoid thromboplastin used here retained practically all its activity when heated at 70° C. for 15 minutes. Higher temperatures were not tried.

Nolf[21] showed that the apparent heat-lability of a clotting factor varied considerably with the circumstances; and the experiments of Morawitz[19] leave questions for further study. Platelets offer an example of the pitfalls encountered. Washed platelets can replace brain lipid in the present test system. But, in contrast to brain lipid, platelets lose much of their acceleratory activity in 10 minutes at 70° C.[18] Whether this represents a loss of Howell's thromboplastin or a loss of some additional platelet factor is not yet clear. Although Howell proposed that some heat-labile materials could function like his cephalin preparations, he recognized no heat-labile factor functioning according to the Morawitz concept of thrombokinase. In fact, he denied that such a factor existed.

Thus, heat-lability is not a good criterion for distinguishing thrombokinase from accessory thromboplastins. These factors must be classified according to their respective actions in the clotting system.

The Presumptive Two-Stage Test for Prokinase Activator

With the purified materials now at hand, and with the present methods, the production of thrombin is never appreciably faster than at the start, as illustrated in Figure 1. However, when crude globulin is incubated with calcium, detectable thrombin is produced only after a latent period, as illustrated by the "not seeded" curves of Figures 4a and 4b. Then the amount of thrombin increases along an S-shaped curve, which often rises more sharply than the ones illustrated.

It is apparent that the latent period is concerned with a reaction which takes place before thrombin is produced. By separating the components of the crude globulin, and by incubating calcium with the activator components in one tube, and with the prothrombin in another tube, it has been shown that the relevant preliminary reaction occurred in the activator system and not in the prothrombin. The preliminary reaction has been interpreted as the activation of prothrombokinase, and a detailed three-stage analysis has been used to study this reaction.[16]

Three-stage analyses demonstrating the effects of thrombokinase[15] and lipoid thromboplastin[17] have already been reported. However, the complexity of the procedure has made it advisable to describe the effects in terms of a simpler, though less rigorous, test.

If the latent period in the two-stage test is concerned with the activation of prothrombokinase, then any factor that activates the prokinase should obliterate the latent period, if added in sufficient quantity. As shown in Figure 4a, kinase shortened the latent period at a dilution of 1/110,000 and obliterated the latent period when seeded in larger amount. Comparison with the data in Table 6 reveals that the largest amount of seed kinase could not have exerted much effect by directly activating prothrombin. The seed kinase apparently accelerated the development of more kinase from the crude globulin. This is consistent with the hypothesis that kinase activates prokinase, but other interpretations are possible.

Although the lipid also shortened the latent period, it did not reduce it to less than four minutes, as shown in Figure 4b. The general effect was to increase the slope and raise the height of the curves, with less of a shift to the left. Here, the conclusions are limited by the finding of an optimum at a 1/220 dilution of lipid. As the amount of lipid was increased beyond the optimum, the latent periods became longer.

The results with the two-stage presumptive test do not eliminate the possibility that lipoid thromboplastin is a direct activator of prothrombokinase; but neither do they support that view. Although more complicated interpretations may be entertained, the results are in accord with the view that thrombokinase activates prothrombokinase.

Discussion

The foregoing results can be formulated in terms of factors, all of which had been named by 1912:

	Basic Clotting Mechanism	*Accessory Factors*
1.	Prokinase $\xrightarrow{\text{Kinase}}$ Kinase	Ca++, and ?
2.	Prothrombin $\xrightarrow{\text{Kinase}}$ Thrombin	Ca++, and Thromboplastin {Platelets, Lipid, "Globulin"}
3.	Fibrinogen $\xrightarrow{\text{Thrombin}}$ Fibrin	?

In this system all three basic reactions are enzymatic, and all three precursors are associated with the plasma globulins. Morawitz[19] in 1905, Mellanby[14] in 1909, and Dale and Walpole[3] in 1916 recognized with increasing emphasis that thrombokinase could be obtained from plasma. The concept of the precursor, prothrombokinase, has evolved from that introduced in 1912 by Collingwood and MacMahon.[2] The concept of accessory thromboplastins is derived from those of Nolf[21] and Howell.[10-12] In 1942, Tagnon[22] described a plasma derivative that activated prothrombin in the presence of oxalate. The history of these developments will be reviewed elsewhere.

The present results have shown that thrombokinase can activate prothrombin without the help of either calcium ions or accessory thromboplastins. On the other hand, lipoid thromboplastin and calcium cause little or no thrombin production without thrombokinase.

The kinetics of prothrombin activation,[16] and the effect of varying the amount of kinase are quite consistent with the view that thrombokinase is an enzyme. So is the fact that trypsin can replace thrombokinase, giving results that follow the same pattern.

Ferguson and Erickson[7] were perhaps the first to describe the complementary effects of trypsin and brain lipid. They further proposed, in 1939, to look for "thromboplastic enzymes" as part of the physiological clotting mechanism. Subsequently, Ferguson, Travis, and Gerheim[8,9] brought forth evidence that a "thromboplastic enzyme" occurred in plasma protein fractions. Recently, Travis and Ferguson[23] reported that their enzyme preparations potentiated platelet thromboplastic action. They expressed some doubt that the relevant plasma factor was identical with the enzyme, plasmin. Notwithstanding some differences in interpretation, many of the experimental data from their laboratory and from this one have been mutually confirmatory.

In this laboratory also, it has been found that there are two complementary groups of factors which accelerate production of thrombin. Within each group, one material can replace another. Thrombokinase and trypsin appear to be direct activators of prothrombin. Platelets, brain lipid, and a globulin factor (slaughter-house plasma) belong in the complementary

group of accessory thromboplastins. It may be noted that both thrombokinase and accessory thromboplastin are independently represented in the globulin fraction of slaughter-house plasma. That is to say, there are at least two "globulin factors" that accelerate production of thrombin. One of them functions as thrombokinase, the other as accessory thromboplastin.

As yet, the hypothesis proposes no mechanism for the action of the accessory factors. Neither does it state that they impinge directly on the corresponding basic reaction. It is only known that they influence the net observable result of the basic reaction.

Calcium is known to influence the activation of crude prothrombokinase.[18] This activation follows the course of an autocatalytic reaction. Acceleration of this conversion by a minute amount of kinase is another indication of autocatalysis. The presumptive tests reported here demonstrate that this acceleration can easily be detected with a simple two-stage procedure.

Summary

1. Thrombokinase, as obtained from the plasma globulins, has been contrasted with thromboplastin, as found in the brain lipids.

2. Thrombokinase and thromboplastin proved to be distinct factors, with complementary functions. With the two factors together, thrombin was produced much faster than with twice as much of either factor alone.

3. Thrombokinase alone was sufficient for the activation of prothrombin. Neither lipoid thromboplastin nor ionic calcium was absolutely necessary. Thrombokinase activated prothrombin in the presence of excess oxalate.

4. Lipoid thromboplastin and calcium together were not sufficient for the activation of prothrombin. Thrombin was produced very slowly, if at all, unless thrombokinase was included. Lipoid thromboplastin behaved as an accessory factor in that it accelerated production of thrombin, provided that thrombokinase was present.

5. Although ionic calcium had little effect on the activation of prothrombin by thrombokinase, it was very important for the accessory function of lipoid thromboplastin. By these principles, it was shown that both the prothrombin and the thrombokinase preparations were practically devoid of accessory thromboplastin of the type studied.

6. The early rate of thrombin production was directly proportional to the amount of thrombokinase; it was not directly proportional to the amount of lipoid thromboplastin.

7. Trypsin could be substituted for thrombokinase in systems containing prothrombin, thromboplastin, and calcium; and the early rate of thrombin production was directly proportional to the amount of trypsin. Trypsin also could be substituted for thrombokinase in the presence of excess oxalate.

8. Thrombokinase lost most of its activity within 15 minutes at 60° C. Practically all the activity of lipoid thromboplastin remained after 15 minutes at 70° C. But heat-lability is not a good criterion for distinguishing thrombokinase from all accessory thromboplastins.

9. Thrombokinase, in minute amount, obliterated the latent period in a presumptive two-stage test for prokinase activator. Lipoid thromboplastin did not obliterate the latent period.

The foregoing results are consistent with a theory which, as yet, is limited to factors which had been named by 1912. The theory offers an historical perspective, and the results provide an experimental basis for the consideration of proposed new factors.

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DIFFERENCES IN RESPONSE AMONG MICE OF FIFTEEN INBRED STRAINS TO THE SUBCUTANEOUS INJECTION OF METHYLCHOLANTHRENE*

LEONELL C. STRONG

In a previous report,[1] five inbred strains of mice (CBA, C_3H, CHI, NH, and JK) were compared in respect to the induction of tumors by the subcutaneous injection of methylcholanthrene. The present study is an extension of that type of experiment and includes all the original inbred strains of this laboratory.[12]

Procedure

Whenever possible, at least one hundred mice of each sex of each of the fifteen strains were used (Table 1). The animals were accumulated slowly and the experiments extended over a period of two years (1942-45). One-tenth cc. of sesame oil containing 1 mg. of methylcholanthrene was injected into the right flank of all mice at the age of 60 ± 4 days. As a rule 5 to 8 mice were kept in a box and the sexes were separated in order to prevent breeding. However, in several instances before the sexes

Table 1

Number of Mice of the Fifteen Strains Used

	No. of mice		
strain	*female*	*male*	*total*
A	129	109	238
C_{57}	131	108	239
C	129	59	188
CBA	108	117	225
CBAN	118	127	245
C_3H	105	120	225
CHI	113	103	216
$C_{18}I$	109	121	230
F	97	132	229
FC	113	103	216
I	76	97	173
JK	118	118	236
JKL	116	102	218
L	117	131	248
N	106	142	248
Total	1685	1689	3374

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were finally separated offspring were obtained from the methylcholanthrene-injected animals and these mice were continued as untreated lines† for several generations.

The mice were fed with "Nurishmix" and a supplement of mixed grains (oats, wheat, and sunflower seeds) and calf meal pellets. Once a week, each group of mice was also given a small piece (¼ slice) of enriched Wonder Bread and fortified milk to which some cod liver oil had been added (20 cc. / quart of milk). The mice were examined weekly for tumors beginning with the ninth week following the injection of the carcinogen. Past experience had demonstrated that practically no tumors appeared before the sixtieth day. As soon as a nodule seemed to be a definite tumor (estimated by the progressive increase in size of a solid nodule, firmly attached to the skin or underlying tissues), the mouse was killed and autopsied. All tumors were fixed in Bouin's fluid and stained with hematoxylin and eosin. If no tumor appeared, the mice were kept until death occurred, at which time an autopsy was performed and all pathological lesions saved for histological examination.

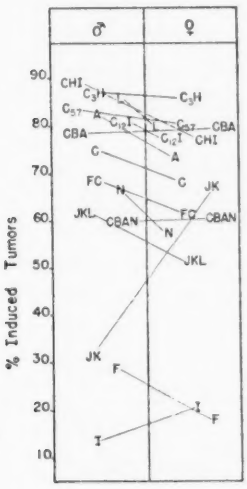

FIG. 1. The ranking of the fifteen strains of mice according to the percentage of mice developing chemically induced fibrosarcomas—data for the sexes are separate.

Brief Descriptions of Strains of Mice

1. A (Strong's albino). The origin of this strain is discussed elsewhere.[9, 12] It is characterized by a high rate of spontaneous mammary adenocarcinomas in females and also by spontaneous lung adenomas in both sexes.

Genetic markers: aa (non-agouti), bb (brown), and cc (albino). This strain, originating in 1920, has been inbred for 103 generations.

2. C (Strong's C). This strain originated in a cross between the A and D (dilute brown) strains in 1920.[12] The C strain was used as the mother one which gave rise to the C_3H, CBA, CHI, and $C_{12}I$ strains.

Mice of the C strain have been used extensively for studies on the influence of genetic and hormonal factors on the origin of double seminal vesicles which they possess,[5] and for studies on the transplantation of a testicular tumor which appeared spontaneously in a male of this strain.

Genetic markers: bb (brown). This strain has been inbred for 86 generations.

3. C_3H—The origin of this strain has been reported previously.[8, 12] The mice are characterized by a high rate of spontaneous mammary adenocarcinomas in both breeder and virgin females. Hepatomas also occur in both old males and females.

Genetic markers: none. The strain has been inbred for 84 generations.

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† One of these lines gave rise to a mutation characterized by dominance. The mutation is characterized by a lightening of hairs when heterozygous and by a lethal anemia when homozygous (N-strain). Another descent (CHI black agouti) for more than 75 generations of inbreeding gave rise to three mutations within five generations —pink eye, piebald, and finally belt (a genetic modification of piebald). Neither the N nor the CHI strains have ever given rise to a spontaneous mutation during a period of twenty-five years of inbreeding.

4. CHI—The origin of these animals from the C strain also has been reported.[13] These mice were used especially by Hooker and Forbes for the bio-assay of progesterone.[9]

5. $C_{12}I$—These mice also originated from the C stock.[12]

Genetic markers: dd (dilution). They have been inbred for 81 generations.

6. CBA—Their origin has been described previously.[10, 13]

The mice were developed by a selection toward longevity: when kept on the old oat meal diet, they would outlive any other mice in the laboratory (average more than 27 months) and would have an extremely low incidence of spontaneous tumors. At

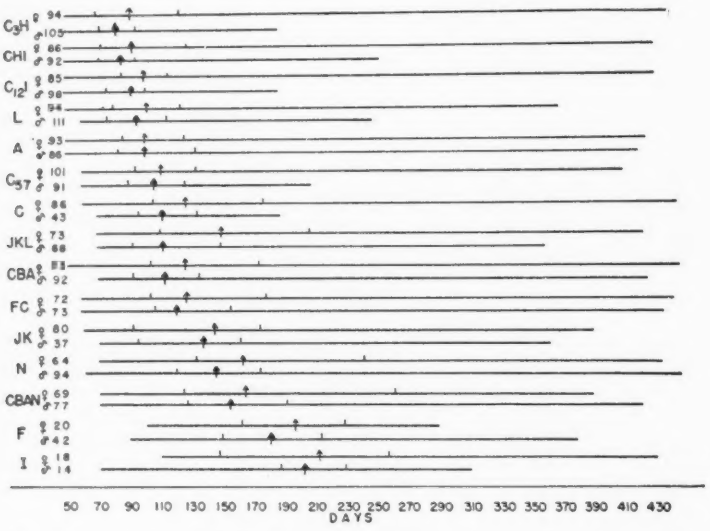

FIG. 2. The data on latent periods for chemically induced fibrosarcomas for mice of fifteen strains (data on sexes shown separately), presented by the quartile method. Numbers of mice developing fibrosarcomas are on left near the left ends of the lines which indicate the time of appearance of the first tumors. The 25% and 75% values are indicated by bars and the 50% (median) values are indicated by arrows. The right ends of the lines indicate the time of appearance of the last tumors.

present, on other diets, the mice have a relatively high incidence of mammary adenocarcinomas in females and hepatomas in both sexes.

Genetic markers: none. They have been inbred for 91 generations.

7. C_{57}—The origin from Little is well known.

Genetic markers: aa (non-agouti). They have been inbred for 28 generations since they were established in the present laboratory.

8. F—The origin has been reported previously.[1] They develop a high incidence of spontaneous lymphatic and myelogenous leukemias. They have been used especially for the study of leukemia by Kirschbaum.[7] These mice are also characterized by small adult body size.

Genetic markers: aa, bb, $c^{ch}c^{ch}$ and ss (low-grade piebald, in most cases only a white face). They have been inbred for 75 generations.

9. I—This is one of the original inbred strains reported previously.[19]

Genetic markers: pp (pink eye) aa, bb, dd, and ss. This strain has been inbred for 63 generations.

The mice are characterized by a high incidence of spontaneous adenomatous hyperplasia of the gastric mucosa. However, the incidence of spontaneous tumors is extremely low. The animals breed well at all times of the year.

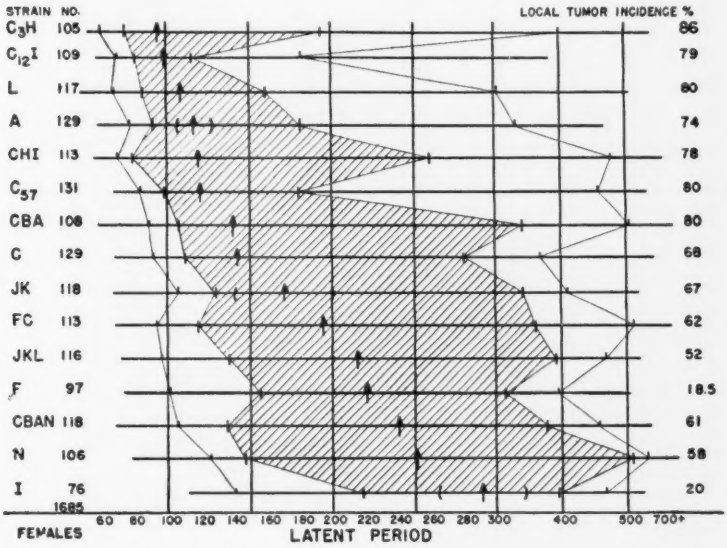

FIG. 3. The data for female mice of the fifteen strains on latent periods for chemically induced fibrosarcomas and time of death of mice which did not develop tumors. The strain, number of mice in each group, the decile, quartile, and median points are indicated. The shaded area includes data between the 25% and 75% values. Percent of local fibrosarcoma incidence is given on the right. Latent period in days after injection to time of death of mice without local tumors is given on the base line. The brackets indicate ± 3 P.E. for strains A, JK, and I. CD determinations are .58 for C_3H; .86 for CBA; .74 for N; and .31 for I.

10. JK—The origin of this strain was reported previously.[12] The males, and the females while nursing, are extremely pugnacious. Except for an occasional lung adenoma the mice seldom develop spontaneous tumors of any kind.

Genetic markers: aa, bb, pp, sese (short ear). The strain has been inbred for 78 generations.

11. L—Origin has been reported by Strong.[18] These mice have very few spontaneous tumors. They have a high incidence of opacity of the lens. They breed well during most of the year but occasionally or seasonally they breed poorly.

Genetic markers: A^wA^w, $c^{ch}c^{ch}$. They have been inbred for 65 generations.

12. N—The origin has been reported previously.[12] The mice develop no spontaneous tumors. They are small in size and breed well for two or three litters. Later sterility is associated with polycystic ovaries.

Genetic markers: aa, bb, dd, pp, ss. They have been inbred for 64 generations.

13. CBAN—This strain was derived from a cross of a CBA female with a male of the N strain. The generations of inbreeding here used were F_{21}-F_{30}.

Genetic markers: aa, ss.

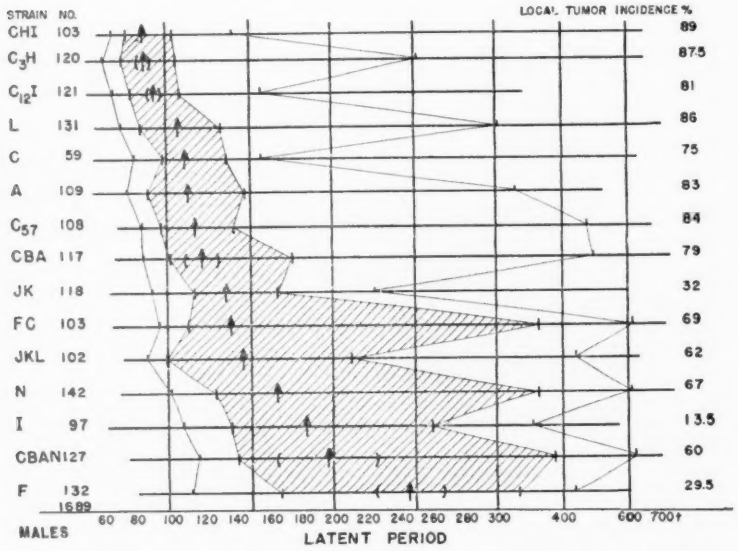

FIG. 4. The data for male mice of the fifteen strains on latent periods for chemically induced fibrosarcomas and time of death of mice which did not develop tumors. Strain, number of mice, earliest appearance of tumor, deciles, quartiles, medians, latest appearance of tumors and percent of tumors are indicated. The brackets indicate ± 3 P.E. for C_3H, $C_{12}I$, CBA, CBAN, and F strains. Latent period in days after injection to time of death of mice without local tumors is given on the base line. CD values are CHI .24; C_3H .18; $C_{12}I$ .15; FC .87; CBAN .62; and F .35.

14. FC—These animals were derived from a cross of an F female and a male of the C_{57} strain. The generations used here were F_{10}-F_{12}.

Genetic markers: aa, bb, $c^{ch}c^{ch}$.

15. JKL—This strain was derived from a cross of a JK female with an L male. The generations here used were F_{10}-F_{12}.

Genetic markers: A^wA^w.

STATISTICAL METHODS

Distributions of latent periods or age at development of induced tumors or death without tumors were generally skewed. Consequently a percentile

analysis, with median, quartiles and deciles is more revealing than the use of means and standard deviations, etc. The distributions are given graphically.

To test the significance of differences between medians, the probable error is calculated from the formula

$$\text{P.E.}_{\text{medium}} = \pm \frac{\text{average deviation of quartiles from median}}{\sqrt{n}}$$

This method has been used for the analysis of biological variability by Dahlberg,[3] Hollander,[4] and others. A measurement of dispersion was obtained as a coefficient, from the formula $\text{C.D.} = \frac{\text{Av. dev. of quartile}}{\text{median}}$.

Incidences of induced tumors are based on total population of each sex of a strain, with the deduction of mice dying before the end of a minimum latent period (60 days after injection). This early mortality is ordinarily negligible, except in males of the C strain.

There was unavoidably some mortality at various ages, due to accident, illness, and other factors. In four strains, early mortality was high, particularly in males. In strains C, F, and I it is impossible to say whether the cause of death was the treatment with methylcholanthrene or simply natural weakness. In strain JK the early mortality of males seemed to be rather a matter of pugnacity—fighting resulting in many deaths (see chart 5).

The incidence of induced tumors is shown in Figure 1. In general, the percentage is lower in females than in males, with the notable exception of strain JK. As noted above, there was a high mortality of JK males at early ages; this fact may account for the unusual incidence of tumors in females of this strain.

The data for latent periods are presented, in two ways, as follows: (i) The distribution of mice, with sexes shown separately, which *developed tumors* (fibrosarcomas) at the site of the injection of methylcholanthrene is given in Figure 2. (ii) The distribution of all mice injected with methylcholanthrene, irrespective of whether they developed a fibrosarcoma or died of some other cause is given in Figure 3 (females) and Figure 4 (males).

A ranking of the strains by median latent period (Fig. 5) gives us the following information: In every strain (except F based on data on all mice injected), the median latent period of the females exceeds that of the males. The sex differences vary from small in A and L to very great in CHI, I, and N. These differences are on the whole statistically significant (notably CHI where mortality is no problem). Furthermore, the general position of a strain in the ranking (short, medium, or long) is similar for both sexes, in spite of the sex differences.

Coefficients of dispersion are generally greater for females than for males,

but also greater for strains in the medium and long ranks, regardless of sex.

If we omit all mice without induced tumors, the ranking is still very much the same for median latent periods, although the numbers involved are in some cases small (Fig. 2).

The data are further analyzed for the rate at which the chemically induced fibrosarcomas appeared in the different strains following the subcutaneous injection of methylcholanthrene. Here the data are cumulative for successive age periods expressed in days (females on Fig. 6; males on Fig. 7).

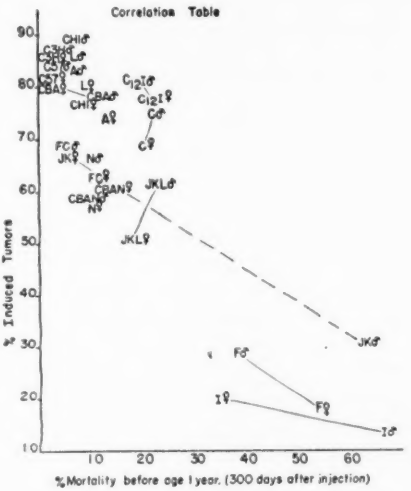

FIG. 5. Correlation table between males and females of fifteen strains of mice for chemically induced fibrosarcomas. Percentage of mortality before one year of age (300 days after injection of methylcholanthrene) is given on the base line, per cent of induced fibrosarcomas on the vertical line.

DISCUSSION

The characteristic differences between mice of inbred strains brought out in this experiment may be attributable entirely to genetic constitutional peculiarities. However, there is little assurance that this is the complete answer. Let us consider some of the difficulties involved in such an interpretation, and, suggest other points of view.

First, the dose of carcinogen was the same for all the mice. But the dose per gram of body weight was not the same. In spite of uniform age, body weight varies considerably, even within pure strains, and there is some difference between sexes. Thus the smaller mice—notably in strains F, I, and N, and, in general, females—received proportionately heavier doses. Since body weight records were not obtained, this facet of the problem cannot be evaluated. But body weight and dosage have been shown to be important variables in other tests (Dunning, Curtis, and Eisen[3]; Strong, unpublished).

Another unlooked-for difficulty is the fact that not all strains were injected at the same time; and even in a given strain, it sometimes required many months or even a couple of years to obtain the required number of mice. Whether there is a seasonal variation of susceptibility or variation due to temporal factors of unknown sort, cannot be determined from the data

available. The possibility of the existence of such a seasonal variation, however, seems unlikely because all mice were kept in an air-conditioned (temperature and humidity controlled) laboratory for the entire time.

Incidence of induced tumors may be considered a useful criterion of the sensitivity of a strain to the carcinogen. However, it was shown by Dunning *et al.* that in rats[3] the parallel relation between dosage and incidence of tumors is upset when high doses of the carcinogen are used, since toxic effects resulted in increased mortality. Something of this sort may explain the results with mice of strains N, I, and F; thus, in spite of a low incidence of tumors and a long median latent period, I-strain males had a 68% mor-

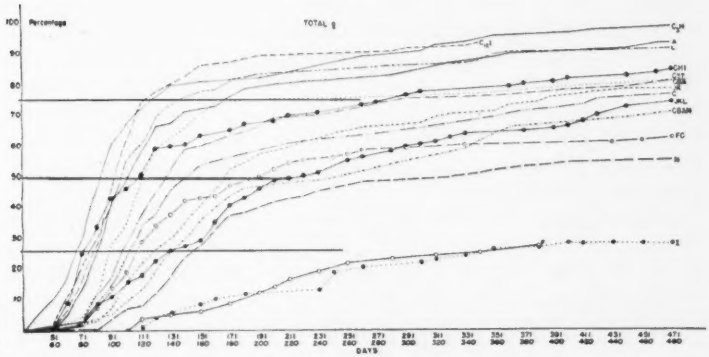

FIG. 6. Data on incidence of fibrosarcoma for female mice of fifteen strains. Average latent periods are given on base line, percentage of mice with induced tumors on vertical line. Data in successive age groups are cumulative; 25%, 50%, and 75% levels are indicated by horizontal lines.

tality before the age of one year (Fig. 5). These mice may seem resistant to carcinogenesis, but may actually be overwhelmed by the toxicity of the drug.

Mortality is therefore a serious problem which has not yet been controlled adequately. Mortality rates, and, correspondingly, rates for the incidence of tumors for CHI and JK strains in the experiment of Burdette and Strong were considerably different from those in the present study!

But within a group having a very low mortality before the age of one year, there still is a pronounced inverse correlation of median latent period with incidence of tumors. These two criteria then seem to be different aspects of the same type of sensitivity to the carcinogen.

It is interesting to note the increasing dispersion when measured by the coefficient of dispersion, as the median latent period increases (see data on Figs. 2 and 3).

The significance of sex differences in sensitivity to the carcinogen seems undeniable. In most cases, and especially in strain CHI, the females are less sensitive than the male, as judged either by incidence or by median latent period. Only one strain (JK) is markedly exceptional. Here again the mortality complicates the picture. Since in general females are not as heavy as males, the difference shown by most strains in sensitivity is the opposite of what one might expect. Perhaps male hormone promotes the carcinogen's action, or female hormone inhibits it.

No consistent relation of any single marker gene to the results obtained seems evident with the exception of s (piebald). The only strains with this

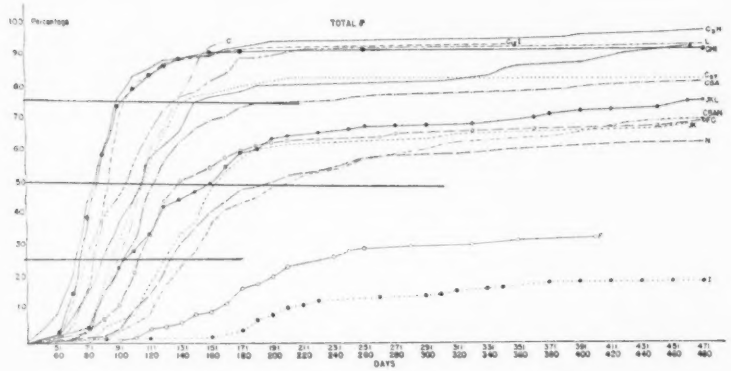

FIG. 7. Data on incidence of fibrosarcomas for male mice of fifteen strains. Average latent periods are given on base line, percentage of mice with fibrosarcomas on vertical line. Data in successive age groups are cumulative: 25%, 50%, and 75% levels are indicated by horizontal lines.

gene are F, I, N, and CBAN. All are in the range of lower incidence. Strong noted that a mutation at the p locus has had no apparent effect on induction of tumors in another strain (the Brp). It is possible that the combination of several mutant genes may be as effective as any single one in influencing susceptibility to tumors induced by methylcholanthrene.

Strain crosses—CBAN, FC, JKL—tend to be intermediate, as a rule, between the parental types but occasionally (JKL ♂, CBAN ♂) exceed the parent in median latent period, and have a slightly lower incidence of induced tumors. Inherited tendencies are present, obviously, but since these crosses are later F generations, we cannot expect any rule to apply.

The present investigation is intended to serve, not as a genetic analysis of susceptibility to the chemically induced tumor, but for the determination of the biological constants associated with the induced tumor upon which a further genetic analysis for the effect of specific genes on carcinogenesis can be made possible.

Summary

One mg. of methylcholanthrene in 0.1 cc. of sesame oil has been injected subcutaneously into 3374 mice (1685 females ; 1689 males) of fifteen inbred strains at 60 days of age. The mice of the different strains possessed different combinations of genetic markers—from wild type (C_3H, CBA, and CHI) to five recessives of the I-strain (ppddbbaass). They developed fibrosarcomas at the site of injection of the carcinogen at different rates. Sex was an important factor in the induction of this neoplasm. The gene s (piebald) was characteristic of all strains with relative resistance to fibrosarcomas. These are the F, CBAN, N, and I. A complete genetic interpretation of susceptibility and resistance to chemically induced fibrosarcomas is complicated by two variables. These are (i) body weight, and (ii) toxicity to the carcinogen. There is a correlation between the coefficient of dispersion and the median latent period for the appearance of the tumors. This coefficient is greater in females than in males. The strains (data for males) can be classified according to susceptibility to chemically induced fibrosarcomas in the following sequence: CHI, C_3H, $C_{12}I$, L, C, A, C_{57}, CBA, JK, FC, JKL, N, I, CBAN, and F.

Two descents were continued from mice injected with methylcholanthrene (N and CHI). These mice gave rise to four mutations. The controls for N and CHI have never given rise to a spontaneous mutation during a period of twenty-five years. Following the appearance of these mutations within five generations of the injection of methylcholanthrene, no more mutations were found. Thus their appearance is associated with the injection of methylcholanthrene and cannot be due to "genic instability."

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CORTISONE AND ACTH IN HYPOPLASTIC ANEMIA*

A. J. ERSLEV, C. K. IVERSON,† AND F. D. LAWRASON‡

For many years hypoplastic or aplastic anemia has been identified with a confusing group of pancytopenias which are refractory to treatment. Recently The Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-forming Organs has redefined the term hypoplastic anemia and discarded the term aplastic anemia.[13] Hypoplastic anemia is defined by this committee as a pancytopenia caused by actual hypocellularity of the bone marrow. This definition probably embraces many diseases with different etiologies but it does place in a single category all cases of pancytopenia due to hypocellularity of the bone marrow.

In most reports of pancytopenias which are refractory to treatment no clear differentiation has been made between cases with hypocellularity and those with hypercellularity of the marrow. This fact makes it hard to draw conclusions concerning the etiology and prognosis of true hypoplastic anemia. There seem to be two distinct types of hypoplastic anemia. One is acute and fulminating and usually is associated with a definite history of exposure to an agent toxic for bone marrow. Avoidance of exposure to this agent not infrequently is followed by spontaneous recovery. The other type is chronic and slowly progressive over a period of months or years. In most of the chronic cases it is not possible to obtain a history of significant exposure to toxins. Recovery from the chronic type of hypoplastic anemia occurs only rarely and unpredictably and apparently is spontaneous rather than due to any specific treatment. No agent has been found which will induce regeneration of the bone marrow in acute or chronic hypoplastic anemia.

Soon after the introduction of cortisone and ACTH in the treatment of a variety of diseases it was discovered that they would often correct a complicating anemia or leukopenia. These findings suggested that cortisone and ACTH had a stimulatory effect on hematopoiesis and might be useful in the treatment of hypoplastic anemia.[5]

The response of normal bone marrow to cortisone and ACTH has not been fully evaluated. Hills[10] and Forsham[7] reported a moderate neutrophilic leukocytosis in normal individuals four hours after a single dose of ACTH but this observation can hardly be interpreted as evidence for stimulation of bone marrow. Administration of ACTH over a period of several days,

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however, produced a marked neutrophilia in two normal volunteers.[10] On the other hand, there have been no reports of significant changes in the number of reticulocytes, red blood cells, and platelets or in the bone marrow of normal individuals following administration of ACTH or cortisone. The eosinopenic effect of cortisone and ACTH probably is unrelated to bone marrow function.[6]

Although cortisone and ACTH have frequently corrected a mild complicating anemia or leukopenia in patients with chronic rheumatoid arthritis[6, 9] or disseminated lupus erythematosis,[15] this response usually has been associated with general improvement in the signs and symptoms of the underlying disease. In patients with severe hematological diseases such as acute leukemia,[9, 11] idiopathic thrombocytopenia purpura,[16] agranulocytosis,[1] and acquired hemolytic anemia,[4] the effect of ACTH or cortisone has occasionally been striking and has produced an almost complete reversal of the bone marrow to normal. However, in both instances it has been felt that the hematological remissions were not due to a direct stimulating effect of the hormones on the bone marrow, but rather to a suppression of the underlying disorder.

Yet it seems possible that cortisone and ACTH may have a direct stimulating effect on bone marrow. Hill and Hunter[9] have reported four cases of refractory anemia with isolated hypoplasia of the erythropoietic tissue where ACTH caused a dramatic remission in one case and delayed, more questionable remissions in the other three. In the discussion of their paper, four similar cases were reported, all of whom responded well to ACTH. A small but definite leukocytosis and reticulocytosis were seen in all of twenty cases of diffuse collagen diseases treated by S. C. Finch.[8] Rosenberg has reported leukocytosis up to 20,000-25,000 in some cases following the administration of corticotrophins.[12] Furthermore, spontaneous adrenal hyperactivity, such as is seen in Cushing's disease, usually is accompanied by leukocytosis,[5] and occasionally by a true polycythemia.[14]

In order to test the effect of cortisone or ACTH on an inactive hypoplastic bone marrow six cases of hypoplastic anemia were treated with these hormones.

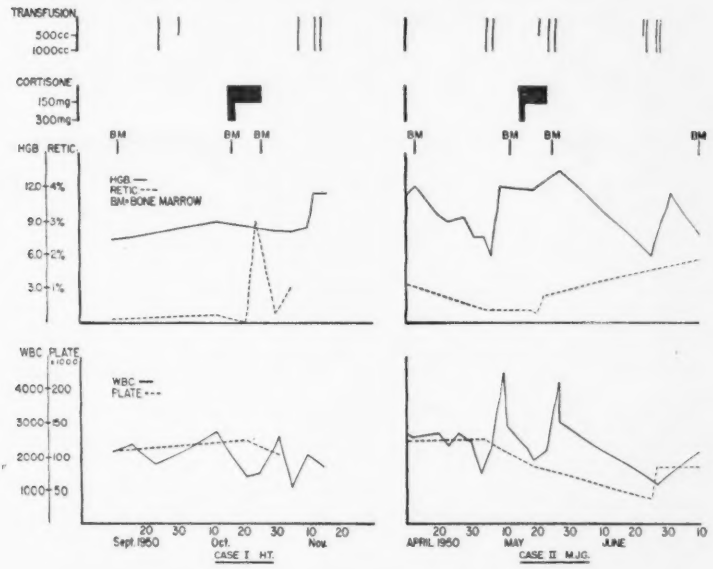
Materials and Methods

The cases presented were admitted for study to the Grace-New Haven Community Hospital during the past year. Each patient had a marked leukopenia, thrombocytopenia, and normochromic anemia. Bone marrow aspirations and biopsies revealed fatty replacement of the marrows, with a relative increase in the number of lymphocytes and plasma cells. Five cases were diagnosed as having chronic hypoplastic anemia, while one case (IV) had acute hypoplastic anemia. In one of the chronic cases (V) and in the acute case (IV) there were histories of brief exposure to subtoxic doses of benzol many months prior to the onset of symptoms. Case VI (reported through the courtesy of Dr. David H. Clement) a young boy, aged seven, also had multiple congenital malformations which suggested a diagnosis of Fanconi's syndrome. However, the pancytopenia had not been present before the age of three (Table 1).

TABLE 1

Case	Age	Type of hypoplastic anemia	Duration of illness prior to therapy	Therapy			Comments
				Drug	Total dosage	Duration in days	
I. H.T.	61	Chronic idiopathic	2½ years	Cortisone i.m.	1800 mg.	10	Short lasting subjective improvement. Further course complicated by a lung lesion, possibly tuberculous. Expired 4 months after Rx. No autopsy.
II. M.A.G.	25	Chronic idiopathic	6 months	Cortisone i.m.	1800 mg.	10	Cerebral hemorrhage 4 months after Rx. Autopsy: Extensive hemorrhages and extreme hypoplasia of the bone marrow.
III. J.G.	54	Chronic idiopathic	4 months	Cortisone i.m.	1200 mg.	10	Hematological condition unchanged 6 months after Rx. Anemia controlled by weekly transfusions.
IV. A.B.	17	Acute ? idiopathic (Minimal exposure to benzol in a photographic laboratory)	3 days	Cortisone i.m.	1500 mg.	12	Acute fulminating course. Cerebral hemorrhage on the 12th day of Rx. Autopsy: Extensive hemorrhages and complete bone marrow aplasia.
V. T.M.	40	Chronic ? idiopathic (Moderate exposure to benzol-containing paint remover)	2 months	ACTH i.m.	440 mg.	20	Hematological condition unchanged 7 months after. Rx. Anemia controlled by weekly transfusions.
VI. R.W.	7	Chronic ? idiopathic ? Fanconi's syndrome. (Multiple congenital abnormalities)	4 years	Cortisone i.m.	900 mg.	7	Cerebral hemorrhage one month after Rx. Autopsy: Extensive hemorrhages, diffuse bone marrow hypoplasia and multiple congenital malformations.

Five patients received cortisone acetate (Merck and Co.) and one patient received ACTH (Armour) (Table 1). Transfusions and antibiotics were administered when necessary. Every other day the patients had leucocyte counts, reticulocyte counts, platelet counts (Rees-Ecker method), and hemoglobin determinations (photoelectric determination of oxyhemoglobin). Bone marrow aspirations (BM on graphs) from multiple sites, with examination of marrow particles squeezed between two slides, were carried out before and after the course of therapy.



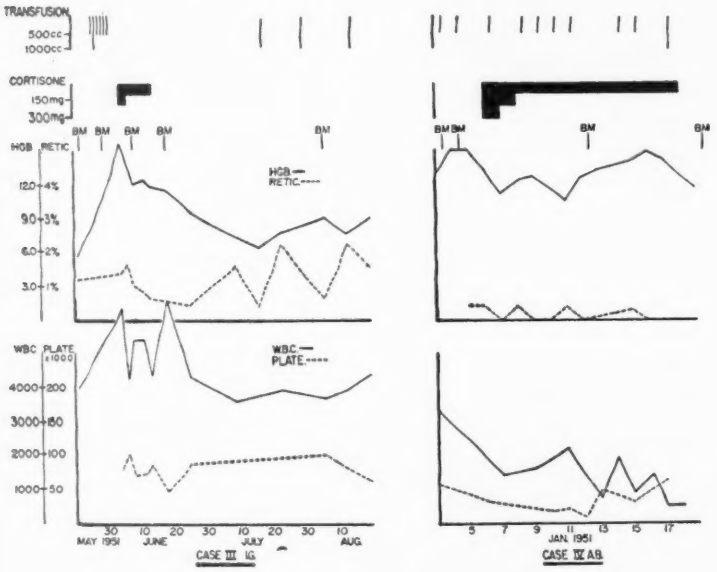
Results

All six cases uniformly failed to respond to cortisone or ACTH. In Case V there was a definite but temporary rise in the number of leukocytes, platelets, and reticulocytes, but repeated bone marrow aspirations and one bone marrow biopsy did not reveal the slightest increase in activity (Graph 3). In cases *II*, *III*, and *VI* a moderate but short lasting rise in the number of neutrophilic leukocytes occurred and in case I there was a rise in the number of reticulocytes (Graphs 1, 2, and 3). However, the bone marrows in all cases remained hypoplastic without exhibiting signs of regeneration. The purpuric tendencies, increased capillary fragilities, or prolonged bleeding times were not altered by treatment in any of the six cases.

Discussion

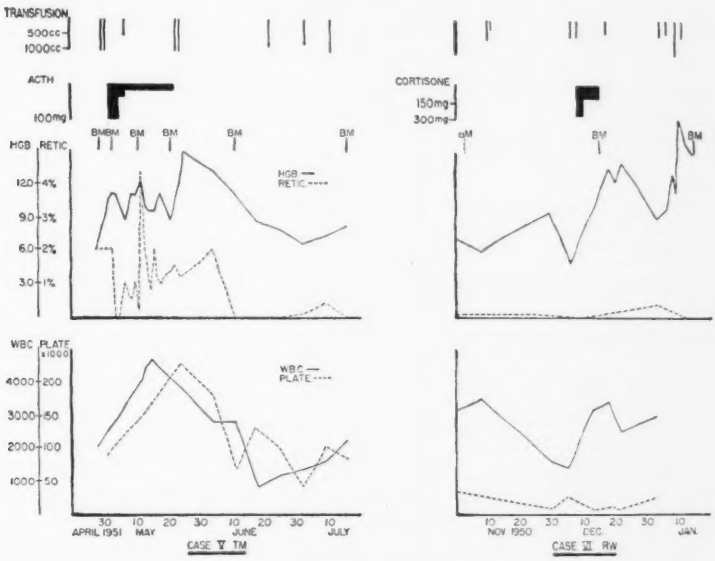
In the last few years cortisone and ACTH have been used more or less empirically in the treatment of many diseases. Hematological remissions

have been reported in a number of these, such as acute leukemia, diffuse collagen diseases, agranulocytosis, refractory anemia, acquired hemolytic anemia and essential thrombocytopenic purpura. A polymorphonuclear leukocytosis and a reticulocytosis have also been observed in many patients during treatment. These observations led to the suggestion that ACTH and cortisone exert a direct effect on hematopoietic tissue and might be of value in the treatment of patients with a deficiency in function of the bone marrow such as that which occurs in patients with hypoplastic anemia.[2]



Hypoplastic anemia is defined as a pancytopenia caused by a hypocellular bone marrow.[13] This hypoplasia is probably due to an eradication or a depression of normal hematopoietic tissue. In the acute type of hypoplastic anemia there is often a history of repeated or excessive exposure to a substance toxic for bone marrow or to irradiation. The acute fulminating course of the disease seems to indicate that a toxic destruction of bone marrow rather than a depression has taken place. In this type it is unlikely that any bone marrow stimulant would be of much therapeutic value. In the chronic type there is usually no convincing history of exposure to excessive doses of an agent toxic for bone marrow. Furthermore, the chronic course of the disease makes it unlikely that acute destruction of the hematopoietic tissue has occurred. The failure to develop extramedullary hematopoiesis and the striking morphological resemblance between the bone marrow in

chronic hypoplastic anemia and normal, inactive yellow bone marrow suggest that the hypoplasia is caused by a chronic depression of hematopoietic tissue. Small doses of various toxins might in some way cause this cellular depression either directly or by interfering with the production of a normal hematopoietic stimulant. In any case, it seems possible that the bone marrow of chronic hypoplastic anemia is merely dormant and potentially able to respond if exposed to the proper stimulating agent.



ACTH or cortisone was administered to five cases of chronic and one case of acute hypoplastic anemia in doses which have induced hematological remissions in other diseases. However, in these six cases the bone marrow failed to respond and the pancytopenia progressed unchecked. The small rise in the number of leukocytes and reticulocytes observed in some of the patients was not sustained or associated with any bone marrow changes and probably was caused by a release of preformed elements into the circulation.

In the literature only brief mention has been made of similar cases of true hypoplastic anemia treated with cortisone or ACTH.[8, 9, 12, 15, 18] The results seem to have been poor except for one case[9] where there was a very suggestive regeneration of the erythropoitic tissue but no improvement of the leukopenia or thrombocytopenia.

Assuming that the bone marrow in chronic hypoplastic anemia is merely inactive and not irrevocably damaged, the results in the five cases presented

here strongly suggest that cortisone and ACTH have no direct stimulating action on bone marrow. The hematological remissions described following treatment with ACTH and cortisone in a number of diseases were probably the result of more normal conditions which prevailed after a primary disorder had been corrected, rather than the result of a direct stimulation of the marrow. The sustained leukocytosis and reticulocytosis observed in some cases treated with these hormones cannot be explained at present.

Summary

Five cases of chronic and one case of acute hypoplastic anemia were treated with cortisone or ACTH for from 7 to 20 days. No significant changes were observed in the hypoplastic bone marrows. Aside from small, temporary changes in the leukocyte and reticulocyte counts, the pancytopenia continued unchecked.

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EFFECTS OF UNILATERAL COLLAPSE OF THE LUNG*

MURRAY Z. ROSENBERG

Since 1882, when Forlanini first offered a rationale for collapse in the treatment of pulmonary tuberculosis, numerous clinical and experimental studies attempting to define the physiological effects of collapse of one lung have appeared. Most of the evidence cited by Pinner[23] supported the clinical impression that unilateral pneumothorax did not interfere with total respiratory function provided a healthy contralateral lung was present. Coryllos and Birnbaum,[6] Dock and Harrison,[9] Andrus,[1] and Moore[18] presented evidence suggesting a diminution in the relative blood flow to a collapsed or atelectatic lung. Cournand[4] found the resting pulmonary artery pressures in five patients with therapeutic pneumothorax to be within normal limits.

While clinical studies of therapeutic collapse[17, 4] have been complicated by the presence of pre-existing pulmonary disease, experimental studies[1, 6, 9, 18, 19, 20] have been complicated by the artificial conditions of acute experiments on heart-lung preparations or on anesthetized animals, and by the technical difficulties inherent in the production of a true unilateral collapse in experimental animals. Attempts to produce unilateral collapse in experimental animals have been all but abandoned since 1924 when Snyder[22] and Matas[16] showed that, because of bilateral equalization of intrapleural pressure in dogs and other animals with thin mediastinal septa, a unilateral pneumothorax cannot produce unilateral collapse as it does in humans.

The purpose of the present study has been to investigate by modern methods the effects of a true unilateral collapse of the lung on the oxygenation of the blood, on the respiration and circulation of each lung, on the intrapleural pressure, and on the blood pressures of the lesser circulation. The experiments were done upon unanesthetized dogs under conditions of rest or moderate exertion.

METHODS

A technique of compressing a lung in a bag of silk mesh has been described by Van Allen and Wang.[24] To circumvent the difficulties encountered by previous investigators in the production of unilateral collapse by pneumothorax, an operation was devised for the maintenance of the left lung of the dog in a collapsed state by enveloping it with a cotton mesh (bridal veil). Complete collapse without compression was obtained by removing positive pressure respiration during thoracotomy and wrapping the contracted lung snugly with bridal veil which was then sutured to itself at suitable

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points before restoration of positive pressure. Polyethylene flanged cannulae prepared in the manner described by Harrison and Liebow[11] were then sutured in place, one to the adventitia of the main pulmonary artery and the other to the wall of the left auricle.

Studies of arterial oxygen and carbon dioxide contents and carbon monoxide combining capacities were made on heparinized blood drawn from the femoral arteries of unanesthetized animals. Analyses were performed in duplicate with the Van Slyke-Neill manometric apparatus according to the standard method.[20]

To determine the effect of exercise, dogs were trotted on a leash covering one mile in twenty minutes on an even grade. A treadmill was later used to study the effect of exercise on arterial and mixed venous oxygen saturations and pulmonary artery pressures in two dogs.

Bronchospirometry was performed under nembutal anesthesia using the double-lumen bronchospirometric cannula described by Bloomer *et al.*[3] An open circuit system was used. Expired air was collected from each lung separately in Douglas bags over a period of thirty minutes; gas volumes were then measured with a gas meter and samples were taken for analysis. Oxygen and carbon dioxide percentages were determined in duplicate with the Henderson-Haldane apparatus.[20]

Bronchial air was collected simultaneously from right and left lungs through the bronchospirometric cannula. The method used was essentially that described by Birath.[2]

Direct intrathoracic pressures were measured with a Hamilton or water manometer. The method used was similar to that used by Brill *et al.*[5]

Pressure determinations were made in unanesthetized dogs from pulmonary artery and left auricle with a Hamilton manometer[19] through spinal needles inserted through the skin into respective cannulae. Pressure curves were obtained with a Cournand catheter from the right ventricle in two dogs before, during, and after exercise. Mixed venous blood was also obtained for analysis in two dogs by catheterization of the right ventricle. Mean pressures were determined from pressure curves obtained by planimetric integration over the period of a complete respiratory cycle. Determinations of average systolic and diastolic pressures were made over the same period.

An angiocardiogram and bronchogram were performed on one dog under nembutal anesthesia.

After completion of these studies, the dogs were sacrificed with nembutal and autopsied. Vinylite corrosion casts were made by injecting pulmonary artery, left auricle, aorta, and trachea using the method developed by Liebow *et al.*[12]

Results

A. *Anatomic observations.* Of the eight dogs subjected to thoracotomy all survived the effects of operation with the exception of dog No. 4, which died with an infarcted left lung, and dog No. 8, which died with a tension pneumothorax. In the surviving dogs at autopsy the collapsed lung was the same in size as at the time of operation (Fig. 1). The sac of cotton mesh was filled to capacity and adherent to the visceral pleura; the enveloped lung obviously contained some air.

Vinylite corrosion casts of the lungs of dogs No. 1, 3, and 6 made four, three, and two months, respectively, after operation demonstrated arteries, veins, and bronchi on the collapsed side. The cast of dog No. 1 is shown in Figure 1 in comparison with the inflated lungs of dog No. 7. Major pulmonary arteries of the collapsed lungs as measured in the casts did not differ significantly in diameter from those of the left lung of normal dogs.

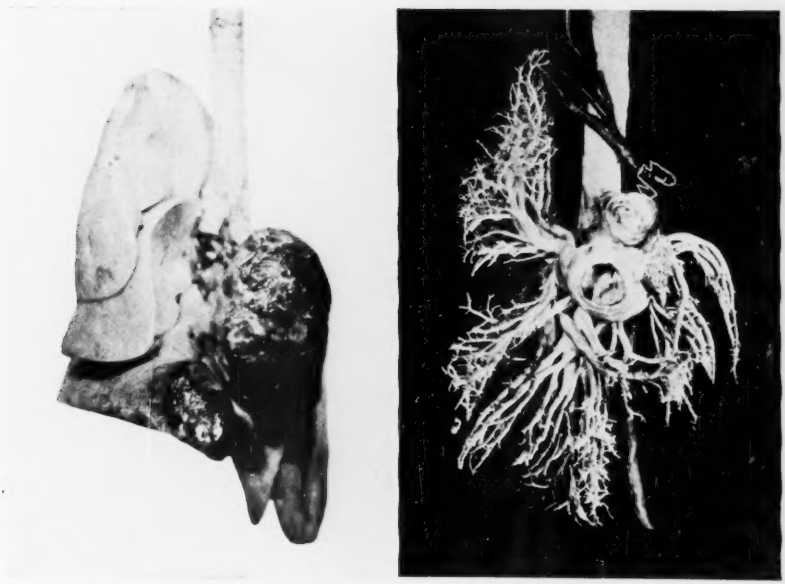

Fig. 1. At the left are shown the lungs of dog. No. 7, outside of the thorax under positive air pressure. Inflation of the left lung is prevented by the wrapping of cotton mesh. At the right is seen the bronchovascular cast made from the lungs of dog No. 1. There is no evidence of bronchiectasis. The bronchial arteries are not significantly enlarged.

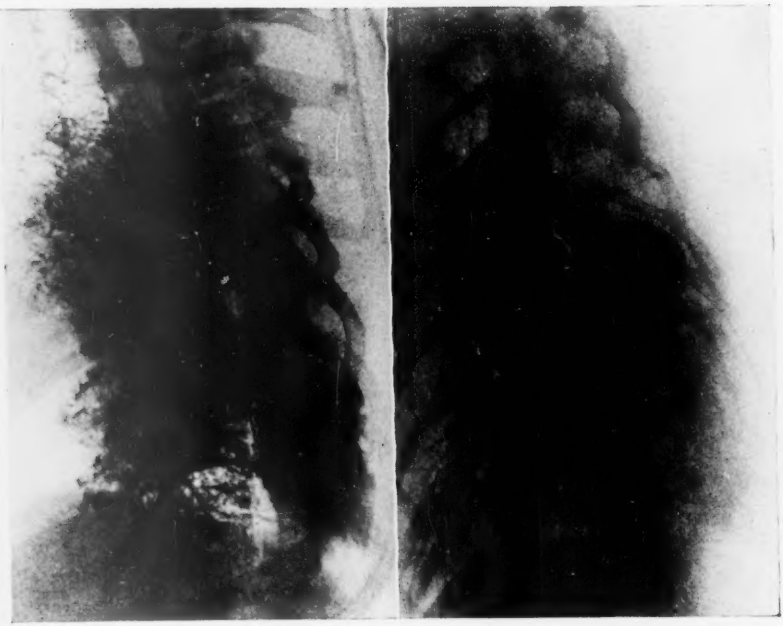

FIG. 2. Bronchogram, dog No. 5, three months after collapse of the left lung. There is no evidence of bronchiectasis, although the bronchi of the left side are shortened and "clumped" together.

FIG. 3. Angiocardiogram, dog No. 5, three months after collapse of the left lung. There is filling of the pulmonary arteries on both sides. Their direction and distribution correspond to their appearance in the cast of another animal (Fig. 1).

No bronchiectasis was demonstrated either in the casts of dogs No. 1, 3, and 6 or in the bronchogram (Fig. 2) of dog No. 5 made in the third postoperative month.

The bronchial arteries were shown to be successfully injected in two casts bilaterally and in a third on the left side only. The marked expansion of

TABLE 1

Dog. no.	*Time*	*O_2 content vol. %*	*CO_2 content vol. %*	*CO capacity vol. %*	*% O_2 saturation*
1	preoperative	22.39	43.62	.....	.....
	1st wk. postop.	19.28	42.11	20.56	93.77
	2d wk. postop.	19.97	47.93	20.61	96.94
	3d wk. postop.	19.28	43.80	20.30	94.97
	3d mo. postop.	22.76	32.86	24.30	93.66
	exercise*	20.50	43.77	21.73	93.92
2	preoperative	19.85	43.51	20.11	98.70
	1st day postop.	23.63	30.19	24.92	94.82
	3d wk.	17.72	36.68	19.23	92.13
	exercise	19.62	40.21	20.31	96.70
3	preoperative	17.00	42.87	17.49	97.19
	1st day postop.	19.67	37.53	20.70	95.02
	3d wk. postop.	17.40	44.17	19.01	91.57
	exercise	21.36	36.65	22.58	94.55
5	preoperative	19.71	35.74	21.36	92.28
	1st day postop.	18.58	36.79	19.87	93.51
	3d wk. postop.	13.52	43.63	14.47	93.43
	exercise	21.11	41.94	22.12	95.43
6	preoperative	19.30	39.62	19.99	96.54
	1st day postop.	16.08	37.80	17.62	91.25
	3d wk. postop.	16.03	42.32	16.86	95.08
7	preoperative	19.39	39.73	20.17	96.13
	1st day postop.	17.64	36.43	19.23	91.26
	3d wk. postop.	15.85	52.09	17.01	93.24
	exercise	18.71	33.75	21.32	87.76

* All exercise determinations were made in the third postoperative months.

the bronchial circulation observed by Bloomer *et al.*[8] after ligation of the left pulmonary artery in dogs was not found.

An angiocardiogram (Fig. 3) of dog No. 5 in the third postoperative month showed that major vessels in the collapsed lung were filled at the same time as comparable vessels in the right lung, this being evidence of the functional patency of vessels in the collapsed lung.

B. *Blood gas studies.* The results of blood gas determinations are shown in Table 1. The mean preoperative resting arterial oxygen saturation was

96.16%; the mean third week postoperative saturation was 93.39%. A low order of statistical significance was found for the difference between these means. There was no apparent trend in postoperative arterial oxygen saturations for the series. Postoperative resting determinations on dog No. 1 in the first, second, and third weeks, and third month likewise showed no trend.

Determinations were performed on five dogs following exercise in the third postoperative month. The mean arterial oxygen saturation for this series was 93.38%. An arterial oxygen saturation determination on dog No. 2 after one hour on a treadmill (three miles per hour) was 94.52%.

C. *Ventilatory and respiratory studies.* The loss of relative respiratory function in the collapsed lung as demonstrated by bronchospirometry (Table 2) was striking. Preoperatively, the left lungs were responsible for an average of 45% of total oxygen uptake; postoperatively, the oxygen uptake of the left lung fell to less than 3% of the total in all dogs.

A rise in the oxygen ventilatory equivalents (liters expired air per 100 cc. oxygen uptake) was noted in the collapsed lungs (Table 2). The range of preoperative values for both lungs and postoperative values for the right lungs were from 1.81 to 3.78; the range for determinations of the left (collapsed) lungs postoperatively was from 5.39 to 9.95. (Kaltreider and McCann[12] found a range of normal values in humans of 1.83 to 3.98.)

In those dogs (Nos. 5, 6, and 7) from whose collapsed lungs ventilatory exchange was too small to permit quantitative bronchospirometry, the gas concentrations of bronchial air gave evidence that some respiratory function was present.

D. *Calculation of distribution of blood flow.* The difference between the preoperative mean O_2 saturation of 96.16% and the third week postoperative mean O_2 saturation of 93.39% may be taken to represent a dilution of arterial blood, returned from well aerated portions of lung, with venous blood returned from unaerated portions of lung. It is of some interest to determine what portion of the total pulmonary blood flow following collapse of one lung does not participate in respiration, i.e., is "ineffective."

The calculation of the distribution of pulmonary flow to aerated and unaerated lung requires a knowledge of the O_2 saturation of blood leaving the right ventricle. Mixed venous blood was obtained postoperatively in dogs numbered 2 and 7 by catheterization of the right ventricle in the unanesthetized animal, and O_2 saturations were found to be 79.43 and 71.50% respectively in these dogs, with an arterial O_2 saturation of 96.43 and 92.13 respectively. If the preoperative arterial O_2 saturations of 98.70% and 96.13% in dogs numbered 2 and 7 are taken to represent the saturation of blood coming from well aerated portions of lung, the "ineffective flow"

TABLE 2

Dog. no.	Time	Respiration (cc./min.)				Postexpiratory bronchial air				Ventilation (liters/min.)		Ventilatory equivalent (liters/100 cc. O_2)	
		Right		Left		Right		Left		Right	Left	Right	Left
		O_2	CO_2	O_2	CO_2	% O_2	% CO_2	% O_2	% CO_2				
1	preoperative	52.88	40.22	50.44	48.17	....	...	....	...	1.25	1.29	2.36	2.56
	3d mo. postop.	82.94	78.25	2.23	1.67	11.62	7.32	13.17	5.85	1.62	0.12	1.95	5.39
2	preoperative	64.21	53.30	46.59	36.01	....	...	....	...	1.71	1.09	2.69	2.34
	3d mo. postop.	72.82	66.16	2.31	1.16	12.76	5.00	18.87	2.80	2.22	0.23	3.05	9.95
3	preoperative	41.46	28.69	44.61	34.92	....	...	....	...	0.75	0.87	1.81	1.94
	3d mo. postop.	101.47	94.69	2.03	0.96	11.32	5.61	13.75	5.19	2.28	0.16	2.24	7.88
5	preoperative	57.39	31.36	25.39	16.56	....	...	....	...	2.11	0.96	3.67	3.78
	2d mo. postop.	107.26	104.22	0.00*	0.00*	15.15	4.86	19.72	0.77	3.53	0.00*	...	...
6	preoperative	56.34	44.04	35.36	25.33	....	...	....	...	1.43	0.77	2.54	2.18
	2d mo. postop.	77.21	51.02	0.00*	0.00*	....	...	....	...	1.94	0.00*	...	...
7	preoperative	60.56	48.32	64.43	52.33	....	...	....	...	2.11	1.78	3.47	2.77
	2d mo. postop.	125.68	96.26	0.00*	0.00*	11.92	6.65	13.63	5.52	3.82	0.00*	...	...

* Exchange too small to permit measurement by methods employed.

responsible for the drop in postoperative arterial O_2 saturation can be calculated from the following formula:

$$F_v = 100 \times \frac{Sa_1 - Sa_2}{Sa_1 - S_v}$$

where F_v = % total pulmonary venous flow coming from unaerated lung = "ineffective flow."

Sa_1 = % saturation of blood coming from aerated lung (preoperative arterial O_2 saturation) = 98.70 and 96.13 for dogs numbered 2 and 7.

Sa_2 = % saturation of the mixed pulmonary venous blood postoperatively (femoral artery) = 96.43 and 92.13 for dogs numbered 2 and 7.

S_v = % saturation of the mixed systemic venous blood (right ventricle) postoperatively = 79.43 and 71.50 for dogs numbered 2 and 7.

Substituting known values in the above formula, the "ineffective pulmonary blood flow" in dogs numbered 2 and 7 is found to be 11.1% and 16.2% respectively.

If the "ineffective" flow is assumed to represent blood flowing through unaerated portions of the collapsed lung, the "effective" flow of the collapsed lung (determined by bronchospirometry to be less than 3% of the total pulmonary flow) added to these ineffective flows will represent a maximum flow through the collapsed lungs of dogs numbered 2 and 7 of 14.1% and 19.2% of the total pulmonary flow.

E. *Intrapleural pressure determinations.* The results of determinations of intrapleural pressures in centimeters of water are presented in Table 3. In three dogs pressures were found to be approximately the same on the two sides of the chest.

The mean postoperative intrapleural pressure (all determinations) was —12.3. The mean for the control series C_1 to C_3 was —5.4. The control series C_4 to C_8 was taken from Brill[5] and had a mean of —3.3. The mean for the dogs subjected to operation was significantly lower than the mean for the control dogs.

A definite trend toward increased negativity was observed after postoperative effusions cleared in the third postoperative week. The mean for the first three weeks was —7.4; the mean for determinations after the first month was —14.5. Repeated determinations were made on dogs No. 1, 2, and 3; all showed changes consistent with the trend of the series.

F. *Pulmonary artery pressure determinations.* The results of determinations of pulmonary artery pressures on resting unanesthetized dogs are presented in Table 4. Both the ranges and the means of pulmonary artery mean pressures for the experimental and control series agreed closely, as

did also the mean diastolic pressures and the pulmonary artery-left auricular pressure gradients. Mean systolic and pulse pressures were somewhat higher in the experimental than in the control series.

Right ventricles were catheterized in dogs numbered 2 and 5. Pressure curves obtained in these dogs while running on a treadmill for 60 to 90 minutes respectively demonstrated no significant change in pressure with exercise.

TABLE 3

INTRAPLEURAL PRESSURES (CM. H_2O)

Dog no.	*Time—postop.*	*Left Insp./exp.*	*Left mean*	*Right mean*
2	1st wk.	—10.0/—7.0	—8.5	—8.5
3	1st wk.	—10.0/—5.5	—7.8	.....
1	2d wk.	—9.0/—3.0	—6.0	—6.2
1	2d mo.	—15.5/—11.2	—13.8 (H)	.....
5	2d mo.	—14.3/—10.1	—12.1 (H)	.....
6	2d mo.	—13.0/—8.0	—10.5	.....
7	2d mo.	—28.5/—12.3	—17.7 (H)	.....
2	3d mo.	—19.0/—11.0	—15.0	—14.8
2	4th mo.	—24.8/—13.4	—16.2 (H)	.....
3	4th mo.	—21.0/—10.5	—15.8	.....
			Mean = —12.3	

(H) = determined with Hamilton manometer.

Control		*Control intrapleural pressures*		
Dog no.		*Insp./exp.*	*Mean*	
C_1	.....	—5.0/—3.0	—4.0	.....
C_2	.....	—9.0/—4.0	—6.5	.....
C_3	.....	—9.0/—4.0	—6.5	.....
C_4	.....	—8.4/+0.6	—3.9	.....
C_5	.....	—7.0/—3.6	—5.3	.....
C_6	.....	—5.8/—0.8	—3.3	.....
C_7	.....	—2.8/0.0	—1.4	.....
C_8	.....	—4.5/+0.5	—2.5	.....

C_1 to C_3 observations made in this laboratory.
C_4 to C_8 taken from Brill.[5]

DISCUSSION

Evidence has been presented showing a marked reduction in the blood flow of a collapsed lung unassociated with a rise in mean pulmonary artery pressure. This can only be interpreted as meaning an increased peripheral resistance in the collapsed lung and a decreased peripheral resistance in the contralateral lung. Any explanation for the redistribution of blood flow

following collapse must account for both the direct and compensatory effects of an altered peripheral resistance.

There is evidence for a capillary vasoconstrictive response in the lung to low alveolar air oxygen tensions.[7, 8, 14] Such a vasoconstrictive response in a collapsed lung as a result of local anoxia and a vasodilatation in the contralateral lung in response to increased flow as occurs in normal lungs during exercise may be a sufficient explanation for the circulatory changes observed.

TABLE 4

PULMONARY ARTERY PRESSURES (CM. H_2O)

Dog no.	*Time postop.*	*Average systolic*	*Average diastolic*	*Mean*	*Simultaneous LA*	*Simultaneous IPP*
3	1st wk.	35.0 (31.0-43.7)*	1.4 (0.0-2.5)	10.0	....	—7.8
1	3d wk.	35.0 (31.9-37.4)	17.5 (10.0-21.5)	22.5	....	—8.8
7	2d mo.	54.8 (47.6-60.0)	6.6 (5.7-7.5)	12.4	—5.7	—17.7 (H)
3	3d mo.	30.9 (27.8-33.5)	4.2 (2.1-7.4)	10.4	....	—13.4
5	3d mo.	60.0 (37.3-66.7)	17.4 (13.1-19.8)	29.4	....	.....
2	4th mo.	57.5 (55.0-59.0)	19.0 (12.5-22.5)	27.5	0.9	—16.2 (H)
Mean for series		45.5	11.0	18.7		—11.6
Control Dog. no.						
C_1	.....	37.0	10.0	25.0	—2.0	.....
C_2	.....	34.0	17.0	29.0	8.5	.....
C_3	.....	37.0	8.5	17.0	....	.....
C_4	.....	37.0	—1.0	10.2	....	.....
Mean for series		36.2	8.6	17.8		

LA = Mean left auricular pressure.
IPP = Mean intrapleural pressure.
(H) = Intrapleural pressure determined with Hamilton manometer.
* Ranges in parentheses.

The alterations in ventilatory mechanics produced by collapse of the lung cannot, however, be ignored. The pump action of chest and diaphragm motion on the blood flow of a shrunken lung encased in a rigid bag and on the blood flow of a freely expanding, hypertrophied lung can hardly be the same. The collapsed lung during inspiration may suffer inflow stasis as does the small heart of constrictive pericarditis during diastole.

The exaggerated intrathoracic negativity following collapse can be best explained as due to increased stretch placed upon the elastic tissue of the expanding normal lung. That such stress may ultimately produce elastic tissue changes and irreversible emphysema in the compensating normal

lung with eventual increase in intrapleural pressure has been shown by Longacre.[18]

Summary and Conclusions

Unilateral collapse has been maintained in dogs by enveloping the collapsed lung in a bag of cotton mesh. Studies of anatomical changes accompanying unilateral collapse have been made with vinylite corrosion casts, bronchography, and angiocardiography. The physiological effects of unilateral collapse have been investigated in studies of the blood gases of the relative respiratory and ventilatory functions of the two lungs as shown by bronchospirometry, and of the intrapleural and pulmonary artery pressures.

From the anatomic studies, it is concluded that following collapse of a lung major pulmonary arteries are not reduced in calibre and remain functionally patent and that neither marked bronchial artery hypertrophy nor bronchiectasis occurs.

From the physiological studies, it is concluded that:

1. Complete collapse of one lung does not appreciably impair total respiratory function under conditions of rest or moderate exercise even though respiratory function of the collapsed lung is reduced to less than 3% of the total.

2. The blood flow of a lung is markedly reduced by collapse as a result of either a capillary vasoconstriction or an alteration of ventilatory mechanics or both.

3. When one lung is maintained in a collapsed state, intrapleural pressure falls as the volume of the contralateral lung increases.

4. Following complete collapse of one lung, the mean pulmonary artery pressure remains within normal limits under conditions of rest or moderate exercise.

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SUPPLEMENTARY OBSERVATIONS ON THE OXYGEN PRESSURE GRADIENT BETWEEN THE MATERNAL AND FETAL BLOODS OF SHEEP*

DONALD H. BARRON AND GEORGE ALEXANDER†

The observations to be presented here on the average oxygen pressure gradient that exists between the maternal and fetal bloods of the sheep, separated by the five tissue layers that form the syndesmochorial placenta, are in the nature of a supplement to data published earlier.[4] In that paper data were presented on the form of the oxygen dissociation curves of fetal and maternal bloods obtained from individuals sacrificed at selected stages in gestation, together with the oxygen capacities, contents, percentage saturations, and estimated oxygen pressures of samples of blood drawn from each of the four placental vessels—the uterine artery, uterine vein or venule, umbilical artery, and umbilical vein—after their exposure by caesarian section. From these data, the differences in oxygen pressure of the bloods of the uterine artery and umbilical vein, and the bloods of the uterine vein or venule and the umbilical artery—that is the difference at the two ends of the parallel cords of the placental capillaries[5]—were estimated.

By virtue of the fact that the maternal and fetal bloods do move through more or less parallel cords of approximated capillaries in the placenta of the sheep, some of the published data can be used for the estimation of the changes that occur in the oxygen pressure in the fetal and maternal bloods as they move through the placenta, if some assumptions are made with regard to the flow of the blood and the permeability of the capillaries. These assumptions are: (i) that each stream moves through vessels of constant caliber and at uniform velocity, (ii) that the permeability to oxygen of the placental capillaries and the intervening tissues is uniform throughout their length, and finally, (iii) that the volume of oxygen diffusing per unit time from the blood in one region of the maternal capillaries to the blood in a region of the fetal capillaries is proportional to the difference in oxygen pressure between these two regions.

METHODS AND RESULTS

Data necessary for the calculations of the changes in oxygen pressure in the bloods moving through the placental capillaries were selected for seven representative cases sacrificed during the last ninety days of the gestation

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period (see Table 2). The data required include: (i) the oxygen capacity of the fetal blood, (ii) the oxygen contents, in volume per cent, of samples from the umbilical vein and artery, (iii) the difference between these two values or the oxygen taken up by each hundred cc. of fetal blood perfusing the placenta, and (iv) the oxygen dissociation curve of the fetal blood prepared at a carbon dioxide tension of approximately 40 mm. Hg. The same data are required for the samples of blood obtained from the maternal side of the placenta. No correction was made for the change in the affinity of the haemoglobin for oxygen as the carbon dioxide pressure and the pH changed with the movement of the fetal and maternal bloods through the placental capillaries, for the changes in the two curves appeared to be of the same order and in such a direction that they tended to remain separated by the same distance despite the changes in pH.

Our reasoning and method of computation may be illustrated by a consideration of the data for sheep No. 5, sacrificed 120 days after insemination; the oxygen capacity of the fetal blood was 16.3 volumes per cent, the oxygen contents of the bloods of the umbilical artery and vein 1.84 and 8.45 volumes per cent respectively. Accordingly, en route through the placenta, each 100 cc. of fetal blood takes up 6.61 cc. of oxygen. The maternal oxygen capacity was 19.2 volumes per cent and the oxygen contents of the bloods of the uterine artery and vein were 19.1 and 9.0 volumes per cent respectively; the A-V difference on the maternal side of the placenta was 10.1 volumes per cent. If we assume that all of the oxygen lost by the maternal blood in its course through the uterus and placenta is taken up by the fetal blood, we may conclude that blood is moving 1.53 times faster through the fetal side of the placenta than it is through the maternal; 100 cc. of fetal blood take up about 64 to 65 per cent of the oxygen given up by each 100 cc. of maternal blood.

The oxygen pressures in each of the samples may be estimated from their percentage saturation by means of the oxygen dissociation curves prepared with bloods drawn from the fetus and mother; these curves are presented in Figure 1. So estimated, the oxygen pressures in the uterine artery, uterine vein, umbilical artery, and umbilical vein are 100, 44.0, 8.0, and 22.0 mm. Hg respectively. These pressures are indicated on the curves in Figure 1. The pressure difference is 78 mm. Hg at the arterial ends of the parallel cords of placental capillaries and 36 at the venous ends.

With these data at hand, one might assume that 0.02 cc. of oxygen move from the maternal blood to the fetal per mm. difference in pressure per unit time. Then in the unit of time just after the maternal blood enters the placental capillaries, as the pressure difference is 78 mm. Hg, each 100 cc. as it passes this region of the maternal capillary net will lose 1.56 cc. of oxygen, the saturation will fall to 91.5%, and the pressure to 80 mm. point 1 on the maternal curve (Fig. 1). In the same interval, as the column of fetal blood moves at the faster rate each 100 cc. will take up but 65 per

cent of the oxygen lost by the maternal blood, or 1.01 cc. Hence at the beginning of this first time interval the fetal blood must have contained 8.45 - 1.01 or 7.44 cc. of oxygen, and been 46.4% saturated with an oxygen pressure of 21.5 mm., point 1 on the fetal curve. At the beginning of the second unit of time, the maternal blood will have advanced some distance along the length of the capillaries; the difference in oxygen pressure between these two new points or regions is 58.5 mm., points 1 on the maternal and fetal curves (Fig. 1). Assuming the same rate of flow per

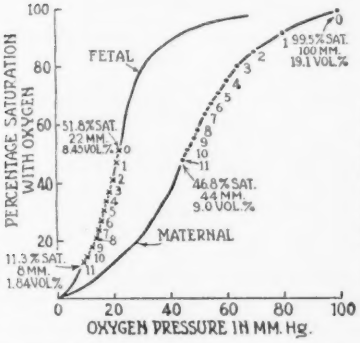

FIG. 1. Sheep No. 5, 120 days' gestation age. Oxygen dissociation curves of fetal and maternal bloods; the curves were prepared at a CO_2 tension of 40 ± 2 mm. Hg.

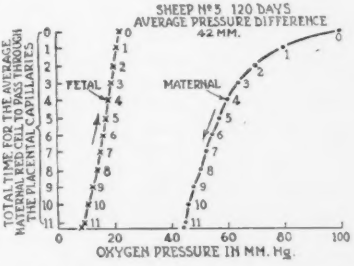
SHEEP Nº 5 120 DAYS
AVERAGE PRESSURE DIFFERENCE
42 MM.
FETAL
MATERNAL
TOTAL TIME FOR THE AVERAGE MATERNAL RED CELL TO PASS THROUGH THE PLACENTAL CAPILLARIES
OXYGEN PRESSURE IN MM. Hg.

FIG. 2. A graph illustrating the estimated changes in oxygen pressure in the fetal and maternal bloods as they pass through the placental capillaries in opposite directions.

mm. oxygen pressure difference, 1.17 cc. of oxygen will be lost from the maternal blood in the second unit of time, the oxygen content will have dropped to 16.4 volumes per cent and the pressure to 70.0 mm. Hg (point 2 on the maternal curve). In the same interval the fetal blood will have acquired 0.76 cc. of oxygen per 100 cc. of blood; accordingly, at the beginning of the interval it must have contained 7.44 - 0.76 or 6.68 cc. of oxygen, and been 41 per cent saturated, with a pressure of 20 mm. Hg,—point 2 on the fetal curve.

The analysis was continued stepwise in this fashion through eleven intervals of equal length and a twelfth of approximately 0.23 of the others, with the results presented below (Table 1 and Figs. 1 and 2); 0.23 is the fraction required for the transfer of the additional oxygen from the maternal to the fetal side to bring the contents and pressures to the observed values at the venous ends of the capillary cords.

The changes in oxygen pressure in the two bloods as they move in opposite directions through the placenta at these arbitrarily chosen time intervals are illustrated in Figure 2; the total time required is of course that required for a small group of red cells or a "fraction" of the blood

stream to move through the maternal side of the placenta. If the area between the two curved lines in Figure 2 is imagined to be a rectangle, with its longer side as *time*, the length of the shorter side will equal the

TABLE 1

	Maternal					*Fetal*			
Interval	O_2 *lost vols. %*	O_2 *content vols. %*	*% sat.*	pO_2 *mm. Hg.*	*Maternal-fetal* pO_2 *difference*	O_2 *added vols. %*	O_2 *content vols. %*	*% sat.*	pO_2 *mm. Hg.*
0		19.12	99.5	100.0	78		8.45	51.8	22.0
1.	1.56	17.57	91.5	80.0	58.5	1.01	7.44	46.6	21.5
2.	1.17	16.40	85.5	70.0	50.0	0.76	6.68	41.0	20.0
3.	1.00	15.40	80.0	64.0	45.0	0.65	6.03	37.0	19.0
4.	0.90	14.50	75.5	60.0	42.0	0.58	5.45	33.4	18.0
5.	0.84	13.66	71.2	57.0	40.0	0.55	4.90	30.0	17.0
6.	0.80	12.86	67.0	55.0	39.0	0.52	4.38	26.9	16.0
7.	0.78	12.08	63.0	53.0	38.0	0.51	3.88	23.8	15.0
8.	0.76	11.32	58.0	50.0	36.0	0.49	3.39	20.8	14.0
9.	0.72	10.60	55.3	48.5	36.5	0.47	2.92	17.9	12.0
10.	0.73	9.87	51.5	46.0	35.5	0.48	2.44	14.3	10.5
11.	0.71	9.16	47.7	45.0	36.0	0.46	1.98	12.1	9.0
12.*	0.16	9.00	46.8	44.0	36.0	0.10	1.88	11.3	8.0

* Time interval .23 of others.

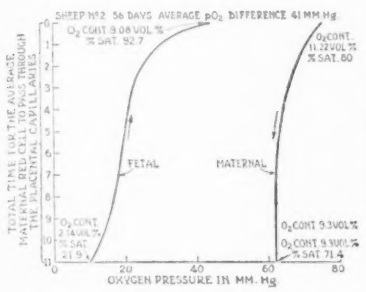
SHEEP Nº2 56 DAYS AVERAGE pO2 DIFFERENCE 41 MM.Hg
O2 CONT. 9.08 VOL.%
% SAT. 92.7
O2 CONT. 11.22 VOL.%
% SAT. 80
FETAL
MATERNAL
O2 CONT. 2.74 VOL.%
% SAT. 21.9
O2 CONT. 9.3 VOL.%
O2 CONT. 9.3 VOL.%
% SAT. 71.4
TOTAL TIME FOR THE AVERAGE MATERNAL RED CELL TO PASS THROUGH THE PLACENTAL CAPILLARIES
OXYGEN PRESSURE IN MM. Hg.

FIG. 3. Sheep No. 2, 56 days. A graph illustrating the changes in the oxygen pressures in the fetal and maternal bloods en route through the placental capillaries.

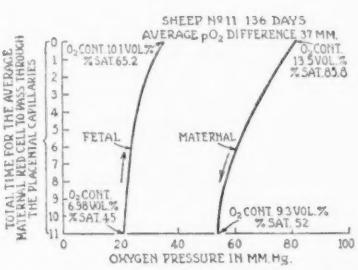
SHEEP Nº11 136 DAYS
AVERAGE pO2 DIFFERENCE 37 MM.
O2 CONT. 10.1 VOL.%
% SAT. 65.2
O2 CONT. 13.5 VOL.%
% SAT. 85.8
FETAL
MATERNAL
O2 CONT. 6.38 VOL.%
% SAT. 45
O2 CONT. 9.3 VOL.%
% SAT. 52
TOTAL TIME FOR THE AVERAGE MATERNAL RED CELL TO PASS THROUGH THE PLACENTAL CAPILLARIES
OXYGEN PRESSURE IN MM. Hg.

FIG. 4. Sheep No. 11, 136 days. The oxygen pressure changes in the fetal and maternal bloods en route through the placenta.

average difference in oxygen pressure between the two bloods as they move through the placenta—in this case 42 mm. Hg. By this same procedure, we have graphed the oxygen pressure changes in the placental vessels in the seven cases, two of which are represented in Figures 3 and 4 and from these

graphs have estimated the average pressure difference between the two bloods. The results are presented in Table 2. Although a maternal oxygen dissociation curve was prepared in the case of sheep No. 2, there was not sufficient blood available to prepare a fetal curve; accordingly, a curve typical of the period prior to 116 days was used for the calculations.

TABLE 2

Sheep no.	*Total age in days*	*Average difference pO_2 maternal-fetal blood*
2	56	41.0
8*	81	43.0
5	120	42.0
70*	126	32.6
3	136	40.0
11	136	37.0
19*	137	48.0

* Twins.

DISCUSSION

There are obvious sources of error in the method we have used for the estimation of the average oxygen pressure difference between the maternal and fetal bloods in these seven cases, but they would not appear to invalidate the conclusion that the values obtained are of the right order. If the values are representative of the conditions *in utero,* then we may infer that the average oxygen pressure gradient across the placenta is of the order of 37-48 mm. Hg; and further, that the gradient does not alter appreciably during the portion of the gestation period represented by our data—56 to 136 days of gestation (full term *circa* 145 days)—this despite the fact that the oxygen requirements of a fetus of 56 days weighing 30 grams would be about 0.24 cc. per minute. In this computation we have used the figure for that stage of 8 cc. per kilo per minute—one that is probably of the right order judging from Barcroft's data (1946). And for a fetus of 136 days, weighing 2.5 kilos, 10.75 cc. would be required, again using Barcroft's data on fetal oxygen consumption at that stage.

If Barcroft's figures on oxygen consumption of the sheep fetus at several stages may be used to estimate the minimal oxygen moving from the maternal to the fetal blood at those stages, then it is possible by combining these with our data on the pressure gradient to calculate the diffusion coefficient of the placenta—the cc. of oxygen crossing it per minute per millimeter difference in pressure on the two sides—at selected stages in gestation. Assuming the oxygen pressure difference between the two sides of the placenta to be 43 mm. the coefficient appears to be about 0.006 at 56 days, 0.12 at 110 days (1250-gram fetus, O_2 consumption 4.2 cc. per kilo per minute) and 0.27 at 136 days. Granted that this coefficient is representative,

the increase may be, and almost certainly is, due in part to an increase in the diffusion surface as the placenta develops to reach its maximum on the fetal side, which occurs at about 110 days, as judged by the volume of blood in its vascular bed.[3] Beyond this stage of development the rise would appear to be due to an increase in the permeability of the tissues that separate the fetal and maternal bloods; and there are histological changes in these tissues, particularly the degeneration of the trophoblast which takes place in this period, that may be correlated with the change in permeability.[4]

Summary

Using data already published, estimates have been made of the average difference in the oxygen pressures of fetal and maternal sheep blood as they pass through the placental capillaries. Data from seven sheep representing different stages in the gestation period were analyzed; the average pressure difference in ewes with singlets (4 cases) ranged between 37 and 42 mm. In twins (3 cases) the range was from 32.6 to 48.0 mm.

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ELECTROMETRICS OF ATYPICAL GROWTH*

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Much has been written and much said about the cancer problem. Large sums of money, large for biological research, but small for investigations in the physical sciences, have been expended in the search for the "cause" of cancer and for its cure. While a considerable number of more or less isolated facts have been discovered, the answer is not yet. Instead of a single factor which gives rise to the atypical growth of malignancy, there seem to be many agents which are possible instigators—constitutional characters (possibly inherited), chemical agents, trauma and mutations, perhaps brought about through endocrine imbalance. The very multiplicity of initiators implies that the answer must be found in some more fundamental property of living systems. It is clear enough that malignancy is a distortion of the characteristic design of the organism. For some reason as yet undiscovered, cells which still retain their capacity for continued multiplication escape from an organismic control. Instead of obeying some set of inherent regulating forces, such dividing units participate in a new growth which, while having definable characteristics, nevertheless departs from the teleological character of normal development. Such considerations make it abundantly clear that the cancer problem is but a special case of the more general central problem of biology, the origin of design or pattern in living systems. The forces inherent in the organism which control morphogenesis, and hence the functions of the whole and its constituent parts, are still unknown. For Aristotle, the regulation was accomplished through the activity, among others, of a "formal cause." Hans Driesch, in modern times, postulated the presence in organisms of a force not amenable to examination by the current techniques of science, which guided and controlled morphogenesis and the continued functioning of the adult. This Entelechy, as he called it, was to be thought of as a supernatural agency outside the present horizons of science. Thus, Driesch aligned himself with the Vitalists who believed in the soul as the directing power. Biologists crucified Driesch for his stand, refusing to admit that there were factors in the living system which were not amenable to rigid description and measurement. Nevertheless, the results of the operation of some sort of powerful, precise forces defining normal and experimentally modified development have rarely been more compellingly described than by this great experimental biologist.

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More recently, Child has invoked physiological or axial gradients as an explanation of the nature of the forces which control the unfolding of structure. That such forces exist is unquestionable. His studies have amply demonstrated the oxygen gradient and its corresponding sensitivity to inimical environmental agents. Just how such a decline in oxygen consumption along the axis of an organism could control rates of cell division and the subsequent differentiation of cells *in situ*, to say nothing of the migration of cells, is not clear.

This is the same sort of problem which is found in genetics. No one questions the existence of genes, or that they can be correlated with morphological characters of the adult, but the forces by which genes impose the genesis of form are no less obscure.

It is clear from the above, and from innumerable other studies, that some sort of control of morphogenesis is present in all living beings. No less obvious is the probability that in atypical growth certain cells, retaining the fundamental capacity for division, escape from normal controls and set up what is in many ways a new organism parasitic on the host. Such release from regulation is clearly not just a local phenomenon, for something happens to the whole organism which is revealed in a number of constitutional changes. Moreover, at some period in the development of the new growth, the basic property of protoplasmic movement exhibits itself and cells wander to new environments. This has been likened to population movements by the late Raymond Dodge, who suggested that cells, like humans, set off on journeys to new pastures seeking more favorable environments.

Such considerations as the above make it clear that not only must consideration be given to the local chemical changes which may be found to accompany atypical growth, but also to the forces which in turn regulate the constant chemical flux. The organism as a whole depends on energy transformations for its continued existence and so does the atypical growth. Energy, however, is a scalar property and is itself indifferent to the direction in which it flows, except that the second law of thermodynamics requires that the flow be such as to increase the entropy of the system. The chemistry of the energy transfers is an absolute requirement for any understanding of the cancer problem, but it is equally important that consideration be given to the fact that in the growth and development of the organism there are forces, in addition to the scalar characteristics of chemical changes, which give direction to the energy flow. It cannot be denied that morphogenesis is directed. This is true of the whole organism and also of its constituent parts. Moreover, the very "directedness" of development implies a necessary "relatedness" of the units of which the system is composed, a relatedness which imparts to the organism that property which makes the whole greater than the sum of its parts.

Considerations, such as those briefly sketched above led, in 1938, to an examination of the electrical properties of malignancies in mice. The experi-

ment was designed to determine whether or not the polarity vectors were altered in atypical growth. For a period of years, a considerable number of mice from the colonies of Dr. L. C. Strong were studied weekly. In all, there were four groups of mice:

A. normal controls; B. a strain of mice known to have a high incidence of mammary cancer, occurring spontaneously; C. a strain of mice prone to atypical growth following the administration of carcinogens; and D. a number of mice from a strain which readily accepted implanted malignant tissue.

From four to eight measurements were made each day and, subsequently, both the magnitude of the potential difference and the polarity were subjected to a statistical analysis. Quite early it became clear that any set of measurements, on any particular day, showed little or no correlation with what had been done to the animal. However, comparison of the groups made it clear that, statistically, the control group showed a different electrical pattern from that found in the animals with spontaneous breast cancer, malignancy produced by carcinogens, or the atypical growth of transplants. There were also statistically significant differences between the spontaneous carcinogenic and the transplanted growth.[9]

Under the conditions of the experiment, individual day-to-day variations in both magnitude and potential distribution were such as to obscure any really significant findings. The results provided an interesting clue but no practical concrete consequences. It was felt, however, that there was some item here of probable significance which needed much more intensive study. Revival of the experiment in the last few years has revealed that the day-to-day care of the mice has to be maintained within rather narrow limits in order to reduce the variability factor. Food, water, temperature, and draughts require careful control. If this is done, the individual variability can be reduced significantly.

A less direct frontal attack on the malignancy problem was undertaken, and aimed at a study of the possible relationships between the vector properties of the electrical phenomena and morphogenesis in a number of forms. During the summer of 1938, through the generosity of F. S. Hammett and the Lankenau Laboratory at Truro, Cape Cod, a study was made of the electrical phenomena associated with the life cycle of Obelia.[18] Here was noted the same kind of phenomena that could be recorded in amphibia[6, 17] and in the chick embryo,[5] namely, that during development there is a steady increase in potential difference along the long axis of the organism until the time when morphogenesis as such, is virtually complete. In Obelia, and it was subsequently confirmed in Amblystoma, during the phases of cell division, the changing potential was an uncomplicated, relatively steady rise in potential difference. However, with the onset of differentiation, the steady climb in potential was replaced by fairly irregular but slow oscillations in potential difference. Subsequently, in a study of the

growth of corn roots, in which moving pictures of corn root growth were made with a simultaneous electrometric recording, the same phenomena were observed of slow but steady increase during cell division, followed by oscillations during differentiation.[19, 21, 23] It was clear from these experiments that the electrical phenomena associated with growth and differentiation were of such a nature as to fulfill the requirement for a set of forces capable of giving direction to morphogenesis. All the studies referred to above are in accord with the original requirements set down by Burr in 1932,[1] and Northrop and Burr in 1935,[2] that an adequate survey of the electrical phenomena of living systems would provide a set of measurements which would, in part, define an electrodynamic field. This field was conceived of as a set of forces in a continuum capable of giving control and direction to all of the unitary components which come within its sphere of activity.

Further evidence of a significant correlate of the importance of relatively steady state standing potentials in morphogenesis was obtained by two quite different kinds of experiments. In the first of these, the electrical patterns of the developing frog's egg were examined. It was found that in the unfertilized egg and in the fertile egg, following through formation of the morula and the gastrula, there could be observed an electrical axis unrelated to any obvious externally observable differences until the appearance of the longitudinal axis of the embryo. The two axes were coincidental. This clearly demonstrates the existence of a measurable constant through development which was related to morphogenesis. It can be argued, therefore, that these potential differences and the pattern in which they are found, provide the direction necessary for a continuous control of the developmental process.[19]

With the coöperation of E. W. Sinnott, similar studies were carried out on the embryos of cucurbits. Four types of gourds were studied in the embryo stage, each of them having its own electrical pattern which, during development, could be correlated with the changing relative growth rates in round, flat, straight, and hook necked gourds. It is well known that the cellular components of these gourds are essentially alike; but in the course of development they are related to each other in different ways. As a consequence of changes in relatedness, the four different gross forms appeared.[22]

One final bit of evidence, that there is a significant correlation between electrical patterns and fundamentally biological properties, appeared as a result of a study of relatively steady state standing potentials in the corn seed,[21, 23] where it was found that the magnitude of the potential difference measured along the long axis of the kernel was a good prediction of relative growth rates in the field and in the genetic constitution of the embryo. High potential seeds grew faster and taller and yielded more than low potential seeds. Thus, there is a possible relationship between available electrical energy and heterosis. Among the strains studied was a single gene mutant. Kernels from this stock had a significantly smaller potential difference than either of the parents and in the field produced dwarf plants. Since it is well

known that genes are significant factors in morphogenesis, another bit of evidence is at hand that potential differences and morphogenesis are related.

If the evidence, briefly reviewed above, is a true picture, a further investigation of the electrometric correlates of atypical growth seems to be in order. It can be argued, therefore, that in atypical growth, with disturbances in the control of normal morphogenesis, an alteration in the electrical pattern, as foreshadowed in the early studies on mice, should be investigated.

Through the very fortunate and enthusiastic collaboration of Dr. Louis Langman, and supported in part by a grant from the United States Public Health Service, a study of the electrometric correlates of malignancy in the generative tract of women was undertaken.[20, 24, 25] In all, more than 900 patients were studied from the Gynecological Service of the Bellevue Hospital. The patients exhibited many varieties of pathological conditions in the generative tract, including malignancy. Eight hundred and sixty of these patients were subjected to statistical analysis. By the clinical evidence, 737 of these were free from malignancy. One hundred and twenty-three, on the other hand, were judged on the basis of complete clinical examination, including biopsies, to be suffering from malignant growth. The voltage gradients, as measured between the posterior fornix and the ventral abdominal wall, showed that the former was positive to the latter in 611 cases, and negative in 126. Of the 123 in the group with malignancy, five patients exhibited positive potential difference in the posterior fornix and 118 a negative one. This gives a chi-square value of 318.5, with 3.841 being the significant number. When this is broken down into age groups, from 21 to 40 and from 40 to 61, the chi-square fact in the younger group was 131.2, and in the older group 80.6. The presence of 56 fornix negative readings in the 509 patients in the younger group can be explained by the fact that all of these patients were still ovulating. It has already been shown in a number of studies that during ovulation the electrical pattern in intact living organisms, from rabbit to man, changes markedly.[2, 4, 7, 8, 10, 12, 15, 18] Furthermore, the existence of five fornix positive readings in the group with malignancy still lacks explanation.

Finally, in the older age group, 53 out of the 198 patients without malignancy gave fornix negative readings. As the study progressed, the incidence of negativity seemed to increase in the period after the menopause. Nevertheless, it is abundantly clear that the great majority of the patients exhibiting fornix negative readings, which could not be accounted for on other clinical grounds, had malignant growths. Conversely, in only five out of 611 instances was the fornix found to be positive in the presence of demonstrated malignancy.

Attention should be called to the fact that this study was carried out in a large hospital service and it is more than likely that although this group was large, it is not a fair sample of the population. The evidence seems clear enough to warrant large-scale investigation of the findings.

All the evidence so far collected make it necessary to reëxamine, under more controlled laboratory conditions, the electrical correlates of malignancy in mice. Again, through the generous coöperation of Dr. H. S. N. Greene, some three hundred mice were intensively studied following implantation of the breast region in the C_3H strain of mice. The implantations made by Dr. Greene in the right axilla were from tumor tissue labelled 4,5,7, and 8 B-PX, and MT8. In addition, foetal and visceral implants were made from normal animals. The technique of measurement which finally evolved was as follows:

The unanesthetized mice were tied down on their backs on a small animal board and the silver-silver chloride electrodes were placed: (i) the refer-

TABLE 1

POTENTIAL DIFFERENCES IN MILLIVOLTS BETWEEN RIGHT AND LEFT AXILLA ON SUCCESSIVE DAYS AFTER IMPLANTATION IN RIGHT AXILLA. SIGN INDICATES POLARITY OF RIGHT AXILLA TO LEFT AXILLA.

	Potential Differences in Days														
Growth	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
None	—1.0	—0.8	—1.0	—0.2	—0.2	—1.0	—1.8	—0.3	—1.0	—1.5	—1.0	—2.0	—1.7	—1.0	—2.0
Foetal	—0.2	—0.1	—1.0	—0.4	—0.5	—2.0	+2.0	—1.0	+1.0	+0.5	+1.0	+1.0	+0.8	+0.2	+1.0
Slow	+0.2	+0.2	—2.2	0	—1.3	+4.4	—1.1	—1.2	—1.8	—2.4	—1.0	—0.6	+0.3		
Rapid	—1.0	—1.0	—3.0	—1.5	—1.6	—3.0	—2.0	—3.0	—3.0	—4.4	—3.0	—2.7	—3.0		

ence electrode in the mouth of the animal, and the exploring electrode, in a drop of physiological salt solution, in the right breast region, and then in the left breast region. Since the left side of the animal was not involved in the implantation, it served to some extent as a control for the right side. The potential differences between the two electrodes were determined by a vacuum tube microvoltmeter with an input impedance of 10 megohms. Since the meter drew less than 10^{-10} part of an ampere, there was an insignificant current drain from the system under measure. As a result, the readings were astonishingly reproducible. Daily determinations were made upon these animals, beginning the day after implantation and continuing for approximately two weeks, or until the implant had achieved something like maximal growth. Normal controls were run to provide a baseline. Since some of these tumors grew rapidly, and others much more slowly, four groups of animals can be reported:

A. The control group
B. The rapidly growing group
C. The slowly growing group
D. The foetally implanted group

In all, 118 animals were studied. The results are graphically represented in Figure 1. It will be noted that if the potential difference measured on the operated side is contrasted with that on the unoperated side, a significant difference appears early, within 24 to 48 hours after implantation in the rapidly growing tumor groups. This difference increased steadily and quite smoothly to reach a maximum of approximately five millivolts on or about the eleventh day, following which it decreased. In the foetal implant group the difference began to appear a little later and reached a peak on approximately the sixth day, following which the potential difference dropped to *zero* and reversed its polarity until the end of the experiment. In the implantations of the slowly growing tumors, potential difference began to emerge on the third or fourth day but reached its maximum of approximately three millivolts on the tenth or eleventh day. From there, until the end of the experiment, the difference in potential fell steadily to *zero*. The control and those without growth showed a variable difference between the two sides of less than a millivolt for the entire experiment. For purposes of clarity, the plotted difference is slightly exaggerated. In all of these measurements the axilla containing the implanted foreign material was negative to the opposite axillary region. It is clear from these findings that the presence of atypical growth in a host organism produces measurable and reproducible electrometric correlates. The rapidly growing tumors developed higher potentials more quickly than the slow growing implants. The foetal tissue started off rather promptly but early reached an electrometric peak and thereafter declined to *zero*, subsequently to appear as a polar reversal which in turn, returned to *zero*. The slow growing implants started late but exhibited an electrometric curve paralleling the essential slope of the rapidly growing tumors, but reached their lower maximum at approximately the same time as the rapidly growing tumors. There is a certain similarity in these plots of potential differences to those found in wound healing.[11, 14] Curiously enough, the time factors are very similar. It is not immediately apparent whether this sequence has any significance. The decline of the potential difference in all three experimental groups is interesting, for it seems to be quite closely correlated with the beginning of necrosis in the atypical growth and in the disappearance of foetal tissue.

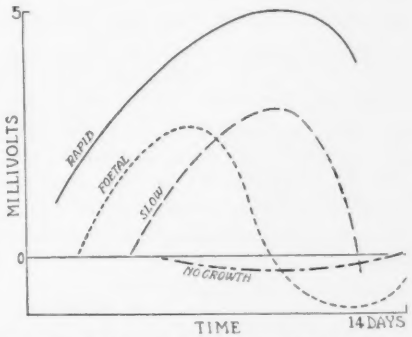

FIG. 1. Smoothed curves drawn from data in Table 1.

Since these experiments were in the nature of fairly acute observations, the presence or absence of any constitutional factor associated with atypical growth was not observed. The success of this last group of experiments was undoubtedly due to the fact that one electrode was placed in the mouth. Thus there was in the mouse experiments an analagous situation to that found in the human patients. In a certain sense it can be argued that in both instances one electrode was in the body cavity and the other on the body surface. As a result, the potential differences were of considerably greater magnitude than when only surface measurements were made. This greater difference made the electrometric correlates considerably more obvious and raised them outside the probable error of determination. Furthermore, it is interesting to note that the atypical growth in both the human cases and in the mice was electrometrically negative to uninvolved tissue.

It would appear from all the evidence seen and reported here that electrometric techniques might be of some value in the early diagnosis of malignancy. However, it is still highly questionable whether the patients used were an adequate sample of the total population. Those drawn from the hospital services were obviously a specialized group. Certainly the procedure is promising, but obviously studies on a much larger scale could well be instituted with a wider selection from the population. Whether or not the procedure would stand up in a general clinical practice can only be determined by further study.

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NOTICE

AMERICAN ASSOCIATION OF THE HISTORY OF MEDICINE

Twenty-sixth Annual Meeting: Columbus, Ohio—April 10, 11, and 12, 1953

Dr. John F. Fulton, President of the American Association of the History of Medicine, has accepted the invitation of the Ohio Academy of Medical History and the College of Medicine of the Ohio State University to hold the next annual meeting of the Association in Columbus, Ohio, in connection with the Sesquicentennial Anniversary of the State. Among the groups collaborating in the commemoration, in addition to the two already named, are the Ohio State Medical Association and the Ohio State Archaeological and Historical Society. The meeting will take place April 10-12, 1953, with the annual dinner scheduled for Saturday evening, April 11th. The headquarters hotel will be the Fort Hayes; the auditorium of the Ohio State Museum has been made available for the meetings. Professor Linden F. Edwards, Department of Anatomy, Hamilton Hall, Ohio State University, Columbus 10, Ohio, is Chairman of the Program Committee.

NUTRITION AND ALCOHOLISM. By Roger J. Williams. Norman, Oklahoma, University of Oklahoma Press, 1951. x + 82 pp. $2.00.

The second sentence of this small monograph reads as follows: "My colleagues and I believe that we have found a basic cause for alcoholism and that by *nutritional supplements* the essential difficulty can eventually be eliminated." The basic cause here referred to is that the alcoholic is characterized by certain peculiarities in his metabolism which raise his requirements for one or more dietary essentials, vitamins particularly. By taking nutritional supplements the essential difficulty is therefore eliminated. The author proposes as a suitable name for this disease concept the word "genetotrophic" (genetics or heredity + trophic or feeding). "It is our conclusion [page 31] based in part on findings to be reported in later chapters, that alcoholism is at least in large part a genetotrophic disease, that is, one which stems from *both* an inborn or hereditary trait *and* nutritional deficiency. The hereditary trait which predisposes toward the disease is the possession of unusually high requirements for certain food elements. These make the individual vulnerable; food as ordinarily chosen is liable not to supply enough, especially when alcohol is consumed; deficiencies result and a craving for alcohol is developed. But if the individual with this hereditary trait is able to get—by any available means—plenty of all nutritional elements he needs, then the disease does not appear, and the hereditary trait which otherwise would be damaging is completely overcome. It is as simple as that."

This reviewer questions whether alcoholism can really be "as simple as that." It is generally recognized that individuals do differ in their requirements for certain dietary essentials, and that some of these differences can be very great. It is this fact which makes it difficult to give any *very precise* figure as the daily requirement for a given vitamin under specified conditions. Figures commonly given are recognized to be rough approximations which can nevertheless serve useful purposes. To conclude, however, from a demonstrated unusually high requirement for one or more dietary essentials by certain alcoholics under study that this is the basic cause of alcoholism seems an unwise and unwarranted over-generalization. This thesis should be subjected to some sort of scientific test. Unfortunately, data from such tests are not included in this volume. Presumably they are to be reported elsewhere.

There is considerable evidence in the literature that many signs of various dietary deficiencies are seen in the alcoholics passing through many of the clinics in our large cities. Jolliffe has pointed out the decrease in incidence of such symptoms in the alcoholics seen in the Bellevue Hospital Medical Center of New York City since the beginning of the flour enrichment program. Although many alcoholics still present themselves to these clinics, fewer and fewer of them give evidence of suffering from dietary deficiencies. This constitutes a good argument against the Williams thesis. Toward this thesis we might take the view that an inherited higher requirement for one or more dietary essentials may *perhaps* be one of the factors predisposing to the development of alcoholism; that it is *the* simple cause, however, cannot be accepted by this reviewer.

GEORGE R. COWGILL

A TEXTBOOK OF GENERAL PHYSIOLOGY. By Hugh Davson. Philadelphia, The Blakiston Company, 1951. xiii + 659 pp. $7.50.

More than ordinary worth marks this textbook by a distinguished English physiologist. Written from the viewpoint of an investigator endeavoring to explain biology in terms of the basic sciences, the book is a common meeting ground for students of zoology, medicine, and the basic sciences.

About one hundred pages of the text are devoted to the structure of living matter, forty pages to energy transformations, and ninety to various aspects of photo-biochemistry. Nearly one-fourth of the book is concerned with excitable tissue and muscular contraction; the transport of ions and solutes is allotted more than two hundred pages. The author elected to omit a specific section on physical chemistry but chose, instead, to explain necessary physical laws in context. Thus, some aspects of colloid chemistry are presented in juxtaposition with the description of the structure of living matter, and the section on energy transformations contains a simple discussion of the First Law of Thermodynamics. This integration, a characteristic of the book, is further exemplified by discussions, wherever possible, of the interrelationship of morphology and function.

The book is written artfully and with intense and meticulous scholarship. Treatment of each subject is lucid, supplemented freely with figures, tables, and footnotes. An extensive bibliography, with references through 1950, follows each chapter.

As pointed out by the author, the book is somewhat restricted in scope, but this should not dampen the enthusiasm with which the book will be greeted. One wishes only that in future editions Dr. Davson may obtain more space in which to include the aborted subjects mentioned in the preface (electrical activity of smooth muscle, aqueous humor and synovial fluid, phototropic phenomena, respiration, and circulation) and perhaps blood and endocrine organs.

J. P. G.

ANNUAL REPORT ON STRESS. By Hans Selye. Montreal, ACTA, Inc., 1951. xxxi + 511 pp. + 133 reference pp. $10.00.

This volume comprises Selye's first annual report of stress, a comprehensive guide to the literature, and his correlation of pertinent facts. Part I, the first of three parts, is entitled "the general physiology and pathology of stress" and is mainly devoted to a discussion of the stress concept, definitions, terminology, stressor agents, and adaptive hormones. In an informal style the author propounds his ideas on the manner in which to use the book; a quick glimpse into the method of production completes the first section. Part II, comprising some four hundred pages, follows under the heading of "special physiology and pathology of stress." Papers relating to intermediary metabolisms, enzymes, skeletal, cardiovascular, and reticuloendothelial systems, kidney, liver, skin, and serologic reactions are summarized. Each subject is presented in an orderly fashion, viz.: (i) stressors, (ii) stimuli influencing the general adaptation syndrome, (iii) diseases of adaptation of the organ or system. Part III is brief and contains Selye's "sketch for a unified theory of medicine."

The unique feature of this first annual report is the 133-page bibliography which is printed on yellow stock and lists the complete references referred to in the text in alphabetic order by authors. Indexing, documentation,

binding, and printing are excellent; footnotes are in a large type face and make for quick and easy reference. Well-captioned, documented photomicrographs, charts, and graphs add to the value of the book.

In the early part of the volume Selye freely interjects his opinions, frankly admits that he cannot write as an impersonal critical correlator of the facts he has chosen to include, but he believes that he has offered an objective guide to the literature. The completeness of this work makes it valuable as a source book and classified index to students and investigators of the stress concept.

J. K. R.

THE MERCK INDEX OF CHEMICALS AND DRUGS. Rahway, N. J., Merck and Co., Inc., 1952, 6th ed. xiv + 1167 pp. $7.50 (regular edition), $8.00 (thumb-index edition).

The Merck Index is too well known to laboratory workers to warrant any detailed description. Suffice it to say that it is principally a handbook describing chemicals of technological or medicinal interest. Many useful tables are handily and succinctly presented.

This edition, which is about one hundred pages longer than its predecessor of 1940, includes several new sections. Among them are a periodic table, a list of more than three hundred organic name-reactions with references, tables of radioactive isotopes, a compilation of standard buffers for calibrating pH measurements, recommended procedures for first aid in poisoning, and weights and logs of elements and groups and their multiples. The largest section, which describes chemicals and drugs, has been considerably expanded both in volume and detail. An entry often includes formulae, synonyms, stability, physical constants and properties of the parent compound and its derivatives, uses, and references to the literature.

These additions have not been made without sacrifice, for much of the information compiled in previous editions has been omitted: the list of clinico-chemical reactions, tests, and reagents (which covered almost four hundred pages in the fifth edition), descriptions of culture media, fixatives, and staining solutions, and the table of oxidation-reduction indicators for biological use. Absent, too, are the list of minerals and their properties and the table of standard solutions.

The tables eliminated, as well as those added, suggest that the latest Merck Index has been groomed more for the chemist than the biologist. This new edition is complementary to the fifth edition rather than a revision of it.

J. P. G.

PATTERNS OF SURVIVAL. By John Hodgdon Bradley. New York, Grune & Stratton, 1952, 2d ed. 223 pp. $3.75.

Many years have passed since the great discoveries of Darwin, Haeckel, and Lamarck, yet man's understanding and interpretation of the development and organization of the living world are still far from being complete or even adequate. The long-standing dichotomy between mechanism and vitalism, the eternal temptation of teleological interpretation, and the conflict as to the relative importance of heredity and environment have come a long way in discussion, but it is hard to see how they could be resolved or dispensed with at this time.

These are but a few of the major problems the author had to tackle in writing this book, and he does justice to both sides of the arguments with unrelenting impartiality. The volume obviously is a survey-book for the non-professional public, nevertheless one would still prefer to have some of the author's own opinions instead of sweeping and often contradictory generalizations in an almost epigrammatic style bestudded with poetic metaphors in the best romantic tradition.

The problems and methods of survival are organized partly chronologically, and partly by the major aims of life, such as love and social organization. This leads to a good continuity in the development of the subject, in spite of the fact that several chapters had previously been published as separate essays.

A highly anthropomorphistic interpretation of Nature and her "actions" may also go some distance to serve this purpose of unity, but it does seem farfetched to visualize Nature as a Communist dictator supervising equal division of protoplasm amongst individuals of a species, or to explain the survival of simple, non-specialized forms of life as an attempt "to achieve racial longevity."

The last several chapters are devoted to man and his place in the living world. The author creates perhaps his most striking and satisfying synthesis here, because after some didactic experimentation he does succeed in placing man in a class now distinct from all others, but well connected to them biologically, in spite of the unavoidable *Deus ex machina*, "the brilliant promise of a brain."

The book should not only make good popular reading, but may well serve as collateral reading to an introductory biology course, giving some of the necessary orientation, with adequate factual examples, to the development of the living world.

N. A. H.

DAUNTLESS ADVENTURER. THE STORY OF DR. WINSTON PETTUS. By Edward H. Hume. New Haven, Connecticut, Yale-in-China Association, Inc., 1952. [x +] 195 pp. $2.50.

This is the stirring record of a most attractive and able man who had been born in China of missionary parents and who had come to Yale, first as an undergraduate in 1929 and then to the School of Medicine in 1933. He made a name for himself as an outstanding student in the college and as one of the most promising men of his year in the Medical School. He attracted the attention of both the Departments of Anatomy and Physiology during his freshman year 1933-34; in his senior year both Dean Winternitz and Dr. Cushing became interested in him and he had discussions with both about his future plans for a life as a medical missionary in China. He was graduated with distinction in 1937, after which, on 23 June 1937, he and Miss Maude Miller were married in Dwight Memorial Chapel.

After an internship at the Presbyterian Hospital in New York he and Mrs. Pettus took a trip around the world, visiting his old home in Peking and then hospitals in ten countries. After that he announced his decision on 27 December 1939 of going out to the Yale-in-China hospital at Changsha for a two-year period or longer if it proved mutually agreeable. Thereafter, he had experience in the interior of China, going by way of Hongkong, Canton, Shaokwang, Hengyang, and Changsha.

Pettus gave everything he had as a medical missionary in China and Dr. Edward H. Hume has drawn a clear and sympathetic picture of this man, his aspirations, and those qualities which distinguished him as a medical scientist at home and abroad. On 23 November 1945 was received the melancholy cablegram from Huangping, China, which read as follows: "Dr. Pettus killed in airplane accident at Huangping Kweichow. Immediately sending men to the scene. Have wired Fulton [Dr. Brank Fulton]. Please cable instructions regarding burial . . . H. C. Chang, Dean." The crash had actually occurred on 18 November.

Pettus thus had only ten years in the active practice of medicine in his chosen field as a medical missionary in Central China. Sir William Osler once referred to John McCrae who wrote the imperishable lines, *Flanders Fields,* as one who was "doubly dead because he died so young." This sentiment applies equally to Winston Pettus, M.D. 1937, of the Yale School of Medicine.

JOHN F. FULTON

A Study of Antimetabolites. By D. W. Woolley. New York, John Wiley & Sons, Inc., 1952. $5.00. xiii + 269 pp.

For many years there has been accumulating experimental evidence leading to the opinion that the biological actions of many substances may be attributed to their interference with essential metabolites. One way that such interference may come about is that the antimetabolite, structurally related to a coenzyme or natural substrate, competes for an enzyme associated with the metabolite. Enzyme activity is blocked, cellular metabolism is disturbed, and functional alterations follow. This thesis is exemplified by the competitive relationship between p-aminobenzoic acid and certain sulfonamides on bacterial growth. Its application has yielded, among other things, several useful therapeutic agents.

The interpretation of a major part of pharmacodynamics and chemotherapy in terms of interference with known metabolites is still in a formative stage. There is, however, abundant evidence for critical scrutiny of this heuristic doctrine that may yet become a far-reaching General Principle in biology. Summarization and classification of the data available are necessary. A review of antimetabolites is especially welcome when written by one of the outstanding workers in the field.

Beginning with the history and development of ideas about antimetabolites, Dr. Woolley presents, in turn, factual knowledge, general aspects of the phenomenon, an hypothesis about the mechanism of action, the spectrum of activity of antimetabolites, their natural occurrence in physiological processes and disease, selectivity of action, applications to chemotherapy, pharmacology, biochemistry, and practical suggestions for synthesis and testing of antimetabolites. Although the monograph is written from a definite point of view, data not explicable by the promulgated theory are presented and discussed. Each subject is carefully analyzed and clearly presented in an admirable scholarly style.

One may question the value of the last chapter in which actual experiments are demonstrated. Perhaps these seventeen pages could be better utilized in an elaboration of the electron shifts accompanying modification of a metabolite, correlating them with their effects on enzyme-antimetabolite interaction and the resultant inhibition of bacterial growth. A comprehensive analysis of this type would be a major step in replacing the present

approach to the synthesis of therapeutic agents, now based on naïve pictorial analogies, with a more rigorously scientific and deductive method. However, this lack, and the absence of an author index, are minor shortcomings of an excellent volume that should have great appeal for students and workers in biochemistry, bacteriology, pharmacology, and physiology.

J. P. G.

FRONTAL LOBOTOMY AND AFFECTIVE BEHAVIOR. A Neurophysiological Analysis. By John F. Fulton. New York, W. W. Norton & Company, 1951. 159 pp. $3.00.

It is always a delight when a new book appears by Dr. Fulton. Until recently, Dr. Fulton was Sterling Professor of Physiology and Chairman of the Department of Physiology in the Yale School of Medicine. He is now Sterling Professor of the History of Medicine in Yale University. He is one of the few readable expositors of science, for he uses words to clarify and not to obfuscate and, by some magic, invests them with charm.

This recent addition to his long list of published works is the substance of the Salmon Memorial Lectures, dealing with frontal lobotomy. It is peculiarly fitting that Dr. Fulton should present this summary, because the original experiments from which the whole procedure was derived, were carried out in his widely known laboratories. The original interest has been kept alive over the years by many devoted associates who developed the carefully planned experimental procedure and made many important contributions. With his usual modesty, Dr. Fulton pays tribute to those who have contributed to the program, but for the interpretation of the results he takes full responsibility.

The major portion of the lectures deals with experimental studies on animals. While much of this work was done in his laboratory, he has not neglected studies completed elsewhere. The last chapter, dealing with lobotomy in man, is a very fair evaluation of the results so far achieved. He calls attention to the fact that as more and more cases are carefully studied, the original radical lobotomy will be, in all probability, replaced by more circumscribed interference with particular frontal lobe mechanisms and their connection with other parts of the central nervous system.

Some twenty thousand patients have been subjected to this dissection of "the matrix of the mind." Beneficial results have been achieved in at least half the cases, some of them dramatically.

The concluding sentence deserves to be quoted: "The possibilities inherent in lobotomy for returning the mentally ill to a happier and more useful existence provide one of the most challenging problems in medicine today."

Much has been learned, but there are many exciting frontiers still to be investigated.

H. S. BURR

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